WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: CyFlow Counter System with CD4 easy count kit and CD4% easy count kit
WHO reference number: PQDx 0350-081-00

CyFlow Counter System with CD4 easy count kit and CD4% easy count kit with product codes **CY-S-3023, 05-8401, and 05-8405**, manufactured by **Sysmex Partec GmbH**, **CE-marked regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 8 August 2018.

Summary of WHO prequalification assessment for CyFlow Counter System with CD4 easy count kit and CD4% easy count kit

	Date	Outcome
Prequalification listing	8 August 2018	listed
Dossier assessment	24 May 2018	MR
Site inspection(s) of the quality management system	11 July 2018	MR
Product performance evaluation	October 2017 to February 2018	MR

MR: Meets Requirements

Report amendments and product changes

This public report has since been amended. Amendments may have arisen because of changes to the prequalified product for which WHO has been notified and has undertaken a review. Amendments to the report are summarised in the following table, and details of each amendment are provided below.

Version	Summary of amendment	Date of report amendment
2.0	Change of labelling in the safety data sheet, label and IFU for 04-4012 Hypochlorite Solution due to added H + P statement and signal word in the safety data sheet of the manufacturer of the raw material; as a consequence of adjustments to regulation on handling substances hazardous to waters (part of CLP Regulation (EC) 1272/2008)	16 October 2019
3.0	Change of storage temperature of the Sheath Fluid (Ref. No. 04-4007) from 2-25 °C to 18-30 °C. Sheath Fluid is an accessory	19 December 2019

	solution for flow cytometers operated in laboratories at room temperature and does not need to be kept refrigerated. In-use and shelf life stability were tested within the temperature range of 18-30 °C. Consequently, a change of labelling material was required. Change in type and material of buffer bottle caps in CD4 easy count kit and CD4% easy count kit due to leakage and insufficient practicability (Corrective action CA-18005). The change involved three steps: 1. Change in Product Immediate container - new closure cap for buffer components 2. Change of manual capping procedure for Machine-controlled capping using an automated production line. 3. Automated production line performs filling and capping.	
4.0	Fulfilment and closure of commitment for prequalification to further invest in a continued root cause analysis of the large negative bias observed between CD4 levels measured on the Sysmex instruments and using Sysmex assays as compared to measurements made using other CD4 measuring assays (e.g. FacsCalibur and MPL/CellMek with PLG).	30 April 2020
5.0	1. Substitution of Triton X-100 as part of the Count Check Beads green reagent produced by Sysmex Partec GmbH based on based on the REACH regulation that prohibits the use of TritonX-100 from 4 January 2021. 2. Updating of the product's labelling address and intended use according to IVDR. Sysmex Partec GmbH legal company address changed from Am Flugplatz 13, 02828 Görlitz, Germany to Arndtstrasse 11 a-b, 02826 Görlitz, Germany. 3. Amendment of the intended purpose according to IVDR of CD4 easy count kit and CD4% easy count kit.	21 June 2021
6.0	Change of product codes of IVDR Class A products (CyFlow Counter, Hypochlorite Solution, Count Check Beads green, Decontamination Solution, Cleaning Solution, Sheath Fluid).	15 August 2022

Intended use

According to Sysmex Partec GmbH, "*the **CD4 easy count kit** is a two-component, quantitative IVD test for subpopulation labelling of lymphocytes in adult venous EDTA whole blood, and subsequent enumeration with a suitable Sysmex Partec IVD flow cytometer. The CD4 T cell concentration is useful to assess the immune and clinical status of patients. It is an indicator for the initiation or follow-up of treatment for people living with HIV, in conjunction with other laboratory and clinical findings. The test is intended to be performed*

by trained healthcare professionals and can be used for both manual sample preparation and automated use with a suitable Sysmex Partec sample preparation system."
The ***CD4% easy count kit*** *is a four-component, quantitative IVD test for labelling of leukocytes and a subpopulation of lymphocytes in adult venous EDTA whole blood, which can then be enumerated with a suitable Sysmex Partec IVD flow cytometer. The CD4 T cell concentration and CD4% of lymphocytes in blood samples are useful to assess the immune and clinical status of patients. They are indicators for the initiation or follow-up of treatment for people living with HIV, in conjunction with other laboratory and clinical findings. The test is intended to be performed by trained healthcare professionals and can be used for both manual sample preparation and automated use with a suitable Sysmex Partec sample preparation system.*

The ***CyFlow Counter, accessory solutions, and assays*** *are intended as aid to diagnosis of immune and clinical status of patients and for monitoring, initiation or follow-up of treatment for people living with HIV. The CyFlow Counter is a manual cell analysis system designed for the determination of the absolute number of CD4+ T lymphocytes and the percentage of CD4+ cells in lymphocytes in human EDTA venous whole blood samples. The use of the CyFlow Counter is limited to healthcare professionals, trained by staff of Sysmex Partec GmbH or authorised distributors.*"

Assay description

According to the claim of Sysmex Partec GmbH, for "*the CD4% easy count kit, an aliquot of an EDTA whole-blood sample is mixed with two antibodies, each conjugated to a different fluorochrome for labelling dedicated cell populations. After a fixed incubation time, the two buffer solutions are added, and the sample is ready for analysis on the CyFlow Counter flow cytometer. The light source excites the fluorescent dye linked with the stained cell and the emitted light is detected while a certain volume of blood sample is running through the instrument. The concentration of the dedicated cell populations is calculated by the integrated software. For further information, please refer to the IFU of CyFlow Counter (CY-S-3022IFUEN).*

For the CD4 easy count kit, an EDTA whole-blood sample is mixed with the antibody conjugated to the fluorochrome in a 1:1 ratio. After a fixed incubation time, the buffer is added, and the sample is ready for analysis on the CyFlow Counter flow cytometer. The light source excites the fluorescent dye linked with the stained cells and the emitted light is detected while a certain volume of blood sample is running through the instrument. The integrated software calculates the concentration of the dedicated cell populations. For further information, please refer to the IFU of CyFlow Counter (CY-S-3022IFUEN)".

Test kit contents

Component	Product code	Test/Kit
Instrument		
CyFlow Counter (Flow cytometer)	CY-S-3023	N/A
Software CyView 2.11	N/A	N/A
CD4 easy count kit		
CD4 easy count kit to count absolute CD4+ T-lymphocytes	05-8401	100 tests
Vial containing CD4 mAb PE	05-8401-01	N/A
Bottle containing no lyse buffer	05-8401-02	N/A
CD4% easy count kit		
CD4% easy count kit to count absolute CD4+ T-lymphocytes and CD4 percentages	05-8405	100 tests
Vial containing CD4 mAb PE	05-8405-01	N/A
Vial containing CD45 mAb PE-Cy5	05-8405-02	N/A
Bottle containing Buffer 1	05-8405-03	N/A
Bottle containing Buffer 2	05-8405-04	N/A
Others		
Count Check Beads green Fluorescent Bead controls for system check (correct volume pipetting)	05-4026	50 tests
Cleaning Solution	04-4017	250 ml
Sheath Fluid	04-4016	5 L, including tab
Decontamination Solution	04-4018	250 ml
Hypochlorite Solution	04-4019	250 ml

Items required but not provided

Item Description	Product code
Sample Tubes 3.5 ml	04-2000
A verified pipette 20 µl fix and pipette tips	please refer to manufacturers/ distributors of pipettes and pipette tips
A verified pipette 100 – 1000 µl variable and pipette tips	please refer to manufacturers/ distributors of pipettes and pipette tips
A verified pipette 10 µl fix and pipette tips (for CD4% assay)	please refer to manufacturers/ distributors of pipettes and pipette tips
Venous blood collection system with EDTA as an anticoagulant	please refer to manufacturers/ distributors of blood collection systems
Stopwatch	N/A

Storage

Store the antibody and buffer reagents in the dark at 2°C to 8°C. Do not freeze or expose the reagents to elevated temperatures and keep them away from direct sunlight.
Store Sheath Fluid (Ref. No. 04-4016) at 18°C -30 °C.

Shelf-life upon manufacture

14 months.

Under the storage conditions mentioned above, both the CD4 easy count kit and the CD4% easy count kit will be stable until the expiration date printed on the kit label.

Warnings/limitations

Refer to the current version of the manufacturer's instructions for use (IFU) and the Safety Data Sheet for a complete list of warnings and precautions.

Prioritisation for prequalification

Based on the established eligibility criteria, CyFlow Counter System with CD4 easy count kit and CD4% easy count kit was given priority for WHO prequalification assessment.

Dossier assessment

Sysmex Partec GmbH submitted a product dossier for CyFlow Counter System with CD4 easy count kit and CD4% easy count kit as per the "*Instructions for compilation of a product dossier*" (PQDx_018 v1). The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO.

The manufacturer's responses to the nonconformities found during dossier screening and assessment findings were accepted on 24 May 2018.

Based on the product dossier screening and assessment findings, the product dossier for CyFlow Counter System with CD4 easy count kit and CD4% easy count kit meets WHO prequalification requirements.

Manufacturing site inspection

A comprehensive inspection was performed at the sites of manufacture (Sysmex Partec GmbH, Arndtstr. 11 a-b, 02826 Görlitz, Germany and Exbio Praha a.s., Nad Safinou II 341, 252 50 Vestec, Czech Republic) of CyFlow Counter System with CD4 easy count kit and CD4% easy count kit in February 2018 as per the "*Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics*" (PQDx_014 v4). The inspection found that the manufacturers had an acceptable quality management system and good manufacturing practices that ensured the consistent manufacture of a product of good quality.

The manufacturers' responses to the nonconformities found at the time of the inspection were accepted on 11 July 2018.

Based on the site inspection, corrective action plan review, and commitments identified and subsequently closed, the quality management system for CyFlow Counter System with CD4 easy count kit and CD4% easy count kit meets WHO prequalification requirements.

Product performance evaluation

CyFlow Counter System with CD4 easy count kit and CD4% easy count kit was evaluated at the Institute of Tropical Medicine (ITM) Antwerp, Belgium, which is a WHO Evaluating Laboratory for CD4 enumeration between October 2017 and February 2018. The performance evaluation was conducted using the WHO evaluation protocol (PQDx_114), which was also approved by the in-country ethical review board in Belgium.

A total of 312 fresh venous whole blood specimens were used to study failure rates, reproducibility (intra-assay variation, inter-assay variation, inter-instrument variation, instrument precision) and agreement with the BD FACSCalibur (BD Biosciences) as the reference method using an antibody panel including CD3/CD4/CD8/CD45 monoclonal antibodies (Multiset, BD Biosciences) with Trucount tubes (Becton Dickinson). Lastly, ease of use was assessed.

The acceptance criteria were as follows: specimen failure should be less than 10%. For reproducibility studies, a percentage coefficient of variation (%CV) should be less than 15% for CD4+ T cell counts of less than or equal to 200/µL and %CV should be less than 10% for CD4 counts of more than 200 cells/µL. Compared to the reference method, the bias should be less than 10%.

Specimen failure, which was defined as failure of the instrument to provide valid results, was found to be 2.2 % for venous whole blood specimens.

Testing of fresh specimens was conducted to assess the ability of the CyFlow Counter System with CD4 easy count kit and CD4% easy count kit to provide reproducible results. The overall CV was less than 5% for CD4 absolute counts and CD4%. Individual CV's per CD4 category were all below 5 % for CD4 T cells, well within the WHO acceptance criteria (<10% and less than15% for CD4 counts below 200 cells/µL).

The inter-instrument precision was generally below 5% for absolute CD4 counts and consistently below 5% for CD4%, well below the acceptance criteria of WHO.

For the intra-instrument precision, all blood specimens showed a %CV less than 5 % for both CD4 counts and CD4% on venous blood specimens.

The average inter-assay variability for whole blood was between 4.0 and 4.2% for CD4 %, while it was between 3.4 and 4.9% for absolute CD4 counts.

The inter-assay variability (day-to-day reproducibility) of the normal control was mostly less than 5% (one exception with 5.2%). The low controls were generally between 5-10%, which is normal for low CD4 counts. In comparison, the variability on FACS Calibur was less than 5%.

The inter-assay variability on Multi Check stabilised blood indicated that both CyFlow Counter instruments had a good inter-assay reproducibility as %CV on normal blood controls and was generally less than 5%. The %CV was higher for low controls, but this was expected as precision is decreasing with low counts (<200 CD4 cells/µL), but the %CV on low counts was generally smaller than 10% and, in two cases, less than 12%, well within WHO's acceptance criteria. In comparison, the %CV on FACSCalibur were less than 5% for normal controls and slightly higher for low controls (5-7%).

Carry-over of the CD4 easy count kit was 0.58%, while for the CD4% easy count kit, it was 0.92%.

Regarding the agreement with the reference method, both kits, CD4 easy count kit and CD4% easy count kit, had a clear tendency towards a negative bias compared to FACS Calibur using TruCount tubes. The relative bias was smaller than 10% for the CD4 easy count kit, thus within the WHO acceptance criteria. The relative bias of the CD4% easy count kit was -3,5%, -10,6% and -11,7% for <200, 200 – 500 and >500 cells/µL respectively. The reason why the CD4% easy count kit had a larger negative bias than the CD4 easy count kit is unclear.
When compared to a dual platform, both kits CD4 easy count kit and CD4% easy count kit also showed a clear tendency towards a negative bias as compared to the dual platform: FACS Calibur CD4 percentages and total lymphocyte counts from Abbott Cell Dyn Ruby haematology analyser. The relative negative bias was smaller than 10% for the CD4 easy count kit, thus within the acceptance criteria. The relative bias of the CD4% easy count kit was larger than -10% for the highest CD4 category.

Two technicians involved in the laboratory study assessed the practical use of the CyFlow Counter system. The instrument was considered easy to handle and relatively simple and straightforward to use. The start-up and closing down procedures were relatively short. The time to prepare and stain a blood sample is relatively short (15 min).

Labelling

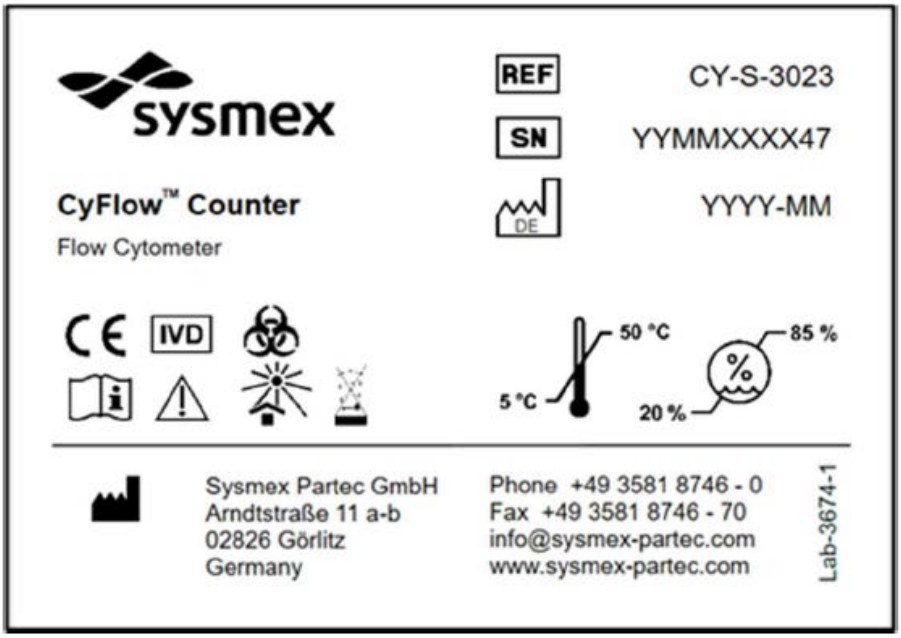
1. **Labels**
2. **Instructions for use**

1. Labels

1.1 CyFlow Counter (Cy-S-3022)

Back Plate Label CyFlow Counter

1.2 Shipping carton label CyFlow Counter



UDI Label

1.3 Easy Count kit (05-8401)- Package labelling

1.3.1. Box label


sysmex
CD4 easy count kit
Contents:
REF 05-8401
LOT 902215
2021-12
100
2°C
8°C
IVD
Sysmex Partec GmbH
Arndtstraße 11a-b, 02828 Görlitz, Germany
(01)04250878900797(17)211200(10)902215

1.3.2 Bottle Label for CD4 mAb PE

1.3.3. Bottle Label for no lyse buffer

1.4 CD4% Easy Count Kit (05-8405)

1.4.1 Box Label

1.4.2 Bottle Label for CD4 mAb PE

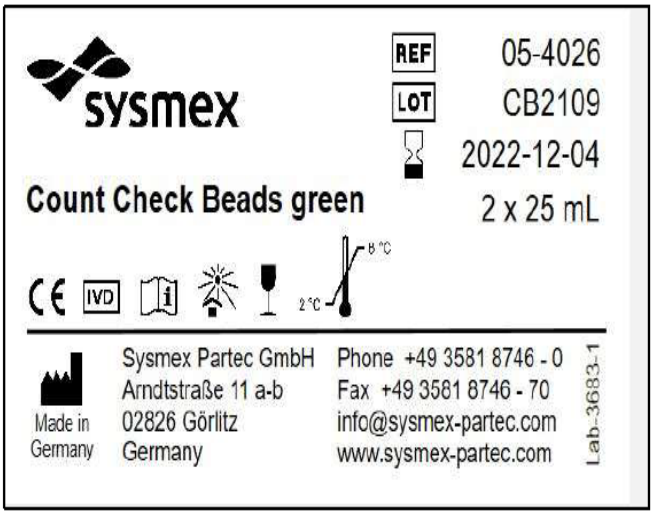
1.4.3 Bottle Label for Buffer 1

1.4.4 Bottle Label for CD45 mAb PE-Cy5

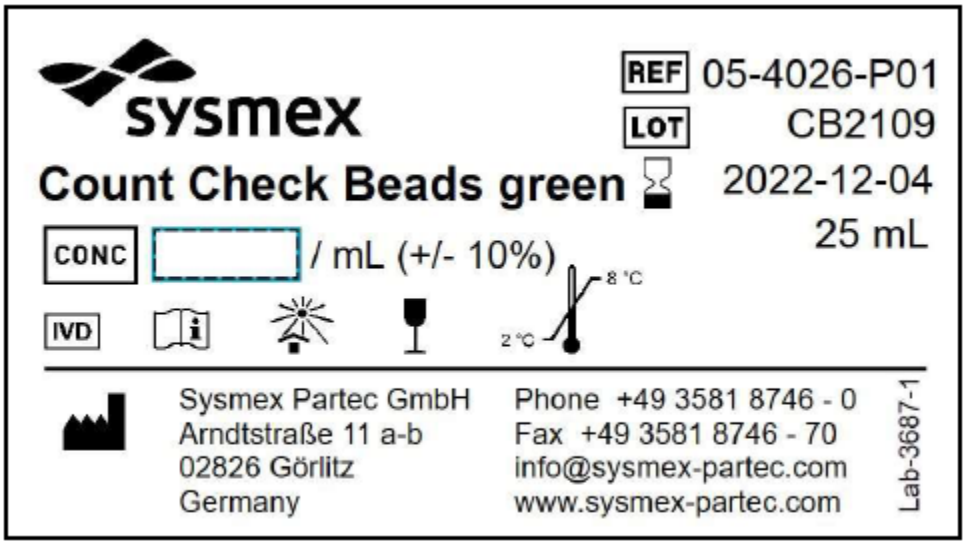
1.4.5 Bottle Label for Buffer 2

1.5 Count Check Beads green (05-4026)

1.5.1 Package label

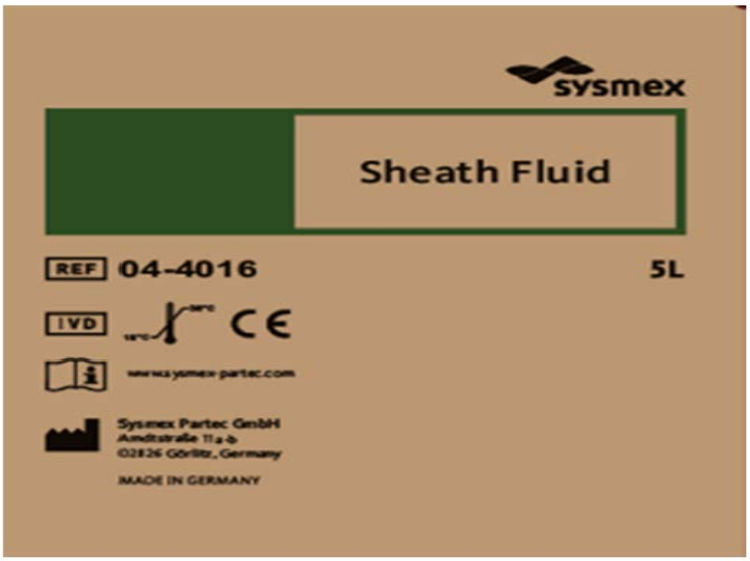


1.5.2 Bottle Label



1.6 Sheath Fluid (04-4016)

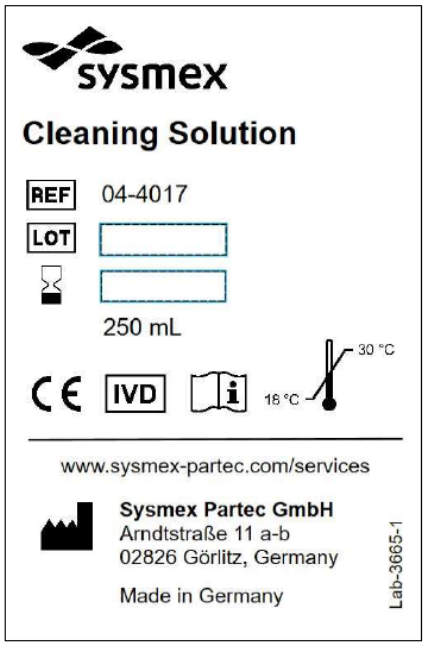
1.6.1 Box 1



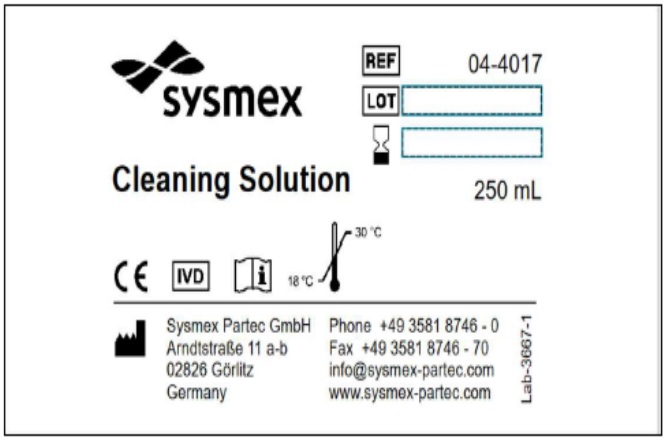
1.6.2 Box Label 2

1.7 Cleaning Solution (04-4017)

1.7.1 Package label

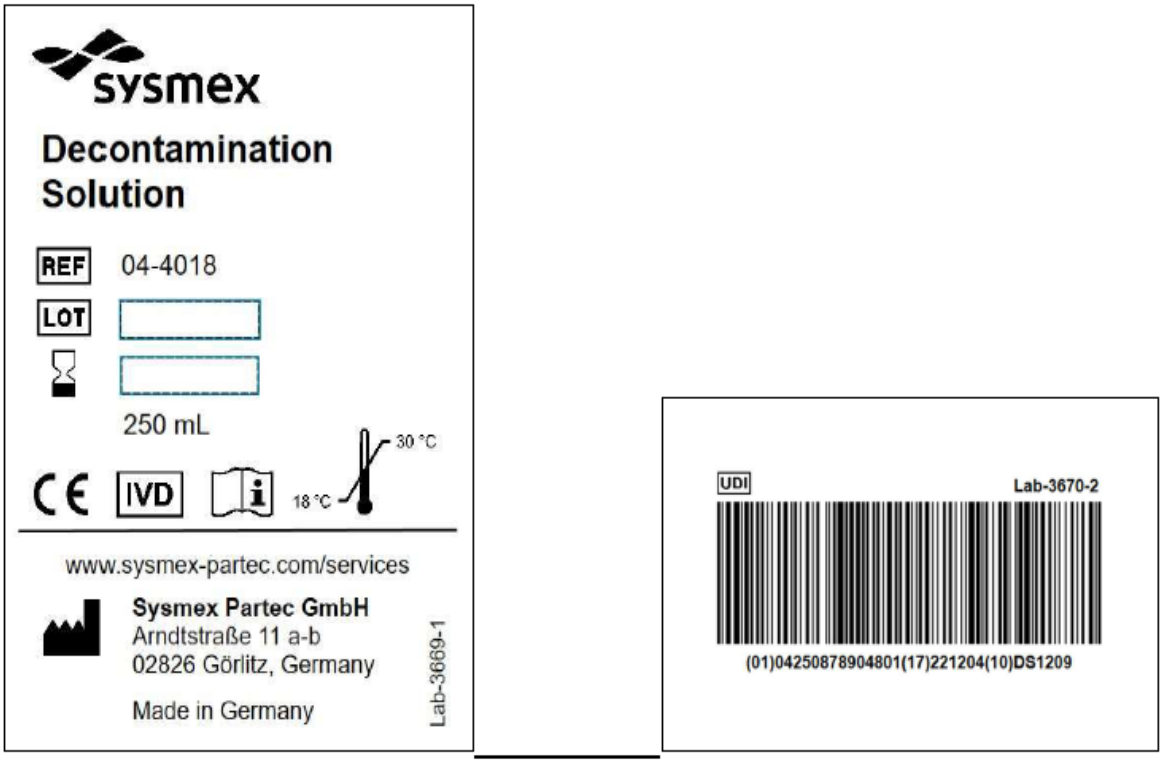


1.7.2 Bottle label

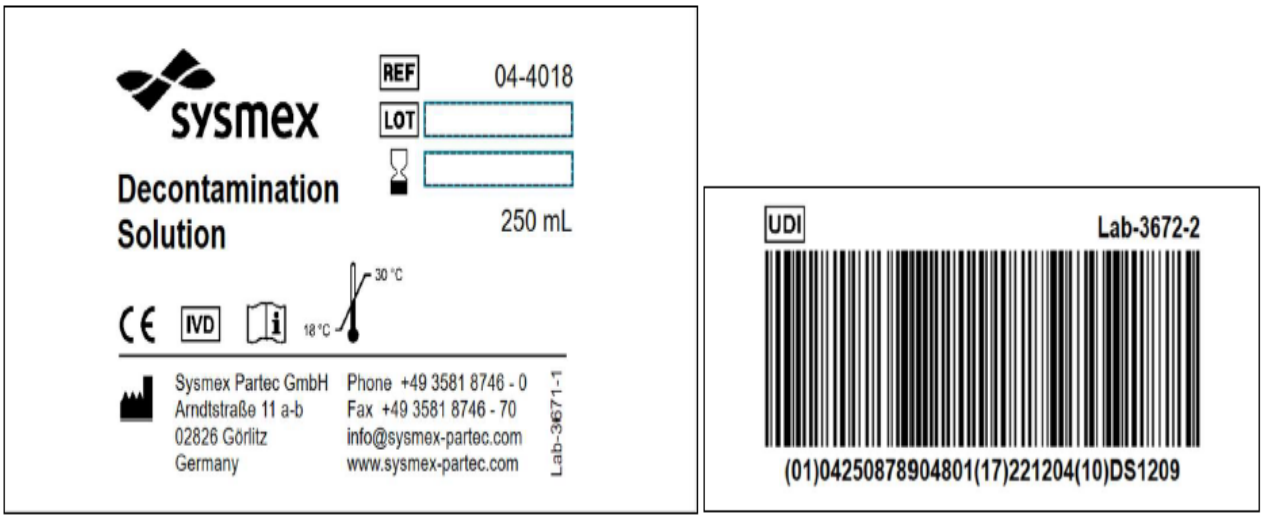


1.8 Decontamination Solution (04-4018)

1.8.1 Package label

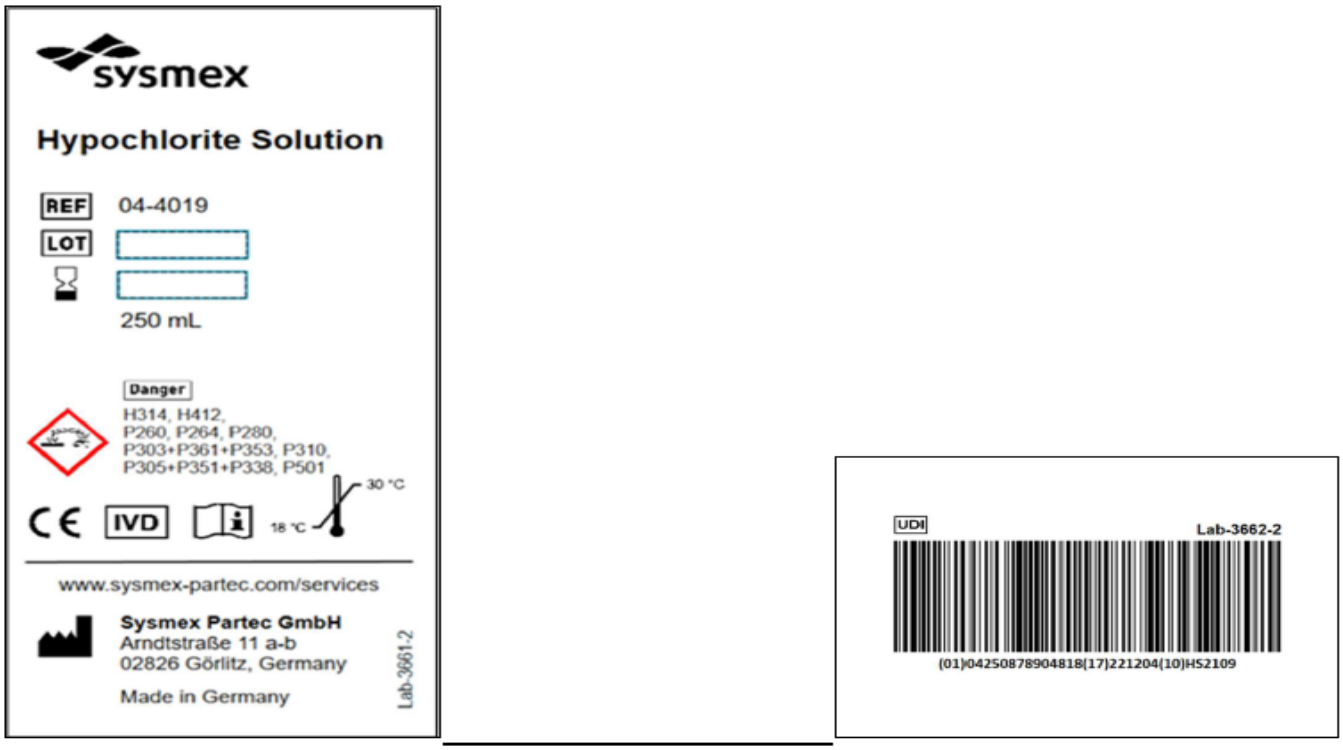


1.8.2 Bottle label

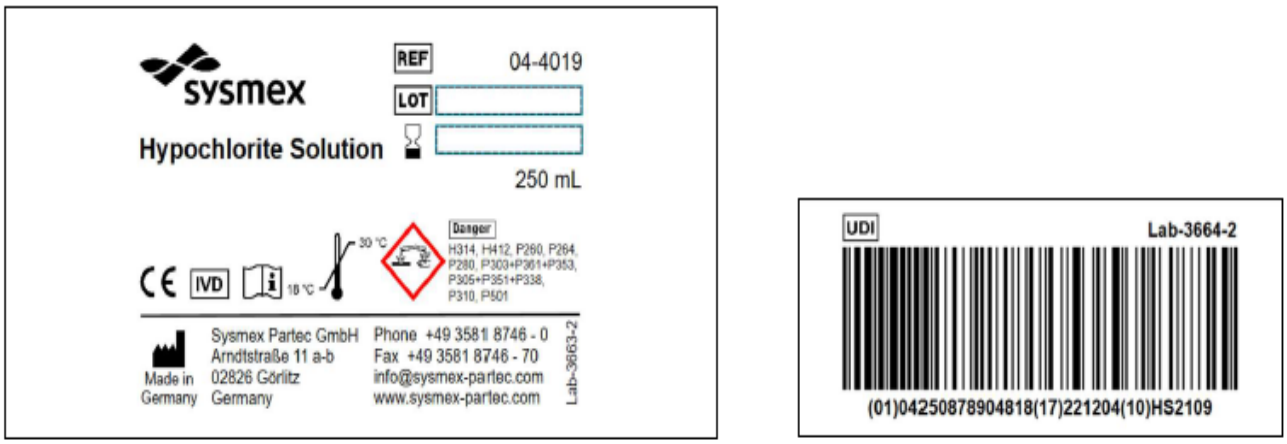


1.9 Hypochlorite Solution (04-4019)

1.9.1 Package label



1.9.2 Bottle label



2. Instructions for use[1]

[1] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

2.1 CD4 easy count kit (product code 05-8401)

IVD CE

Instructions for Use

Identification of the IVD reagent

Product name:

CD4 easy count kit

REF 05-8401

Content:

Vial containing CD4 mAb PE — REF 05-8401-01
Bottle containing no lyse buffer — REF 05-8401-02

1 Specification

Specificity	Human CD4
Isotype	IgG1
Content	100 tests
Fluorochrome	PE
λ excitation (nm)	532 / 488
Emission maximum (nm)	578

2 Intended Use

IVD For In Vitro Diagnostic Use.

The CD4 easy count kit is a manual, two component, quantitative IVD test for subpopulation labelling of lymphocytes in adult venous EDTA whole blood, and subsequent enumeration with the Sysmex Partec CyFlow™ Counter IVD flow cytometer. The CD4 T cell concentration of blood samples is a useful indicator for the initiation or follow-up of treatment for HIV positive patients in conjunction with other laboratory and clinical findings.
The test is intended to be performed by trained healthcare professionals.

3 Principle of the examination method

An aliquot of an EDTA whole-blood sample is mixed with the antibody (CD4) conjugated to the fluorochrome in a 1:1 ratio. After a fixed incubation time, the buffer is added and the sample is ready for analysis on the CyFlow™ Counter flow cytometer.
The light source excites the fluorescent dye linked with the stained cell and the emitted light is detected while a certain volume of blood sample is running through the instrument.
The integrated software calculates the concentration of the dedicated cell populations.
For further information, please refer to the IFU of CyFlow™ Counter (CY-S-3022IFUEN).

4 Storage and shelf life after first opening

1. Storage
Store the antibody and buffer reagents at 2 - 8°C in the dark. Do not freeze or expose the reagents to elevated temperatures and keep them away from direct sunlight.
2. Shelf life after first opening:
Always close the bottle after use and use new pipette tips each time the reagent is sampled to avoid contamination.
Keep the antibody and buffer reagent at 2 - 8°C in the dark. Do not freeze or expose the reagents to elevated temperatures and keep them away from direct sunlight.
Under these storage conditions, the CD4 easy count kit will be stable until the expiration date printed on the kit label.

5 Components

CD4 mAb PE is a murine monoclonal antibody supplied in PBS buffer with 0.2% BSA and 0.09% sodium azide. No lyse buffer is a PBS-based solution containing 0.09% sodium azide.

6 Evidence of deterioration

The antibody and buffer solutions are clear liquids. Do not use the reagents if there is the appearance of any kind of turbidity or contamination.
For questions regarding the performance or quality of the product received, please contact your local Sysmex representative.

7 Precaution and warnings

Reagents contain 14 mM sodium azide as a preservative. The low concentration of sodium azide does not require hazard labelling, but the normal safety precautions for the handling of chemicals must be observed. Refer to the Safety Data Sheet (SDS) for a complete list of warnings and precautions.

8 Additional required equipment

Instrument requirement:	CyFlow™ Counter (REF No.: CY-S-3022)
Required apparatus:	Sample Tubes 3.5 ml (REF No.: 04-2000) A verified pipette 20 µl and pipette tips A verified pipette 100 – 1000 µl and pipette tips Venous blood collection system with EDTA as anticoagulant Stop watch

9 Reagent preparation

CD4 mAb PE (REF No.: 05-8401-01), the antibody reagent is ready to use.
No lyse buffer (REF No.: 05-8401-02), the buffer solution is ready to use.

10 Primary sample collection, handling and storage

WARNING ⚠ *All biological specimens and material are considered as biohazards and should be handled as if capable of transmitting infection. Appropriate safety precautions and handling procedures must be applied in accordance with applicable laws and regulations.*

- The blood sample must be a venous, whole-blood sample, collected with a blood collection system which contains EDTA as anticoagulant.
- The blood sample must be transported in a dark container, protected from light and not exposed to elevated temperatures. A suitable transport container is, for example, a Styrofoam box with cooling material. Best transport conditions would be below 25 °C if transported will not take more than 6 hours after donation. For transport and storage time more than 6 hors, blood must be kept at 2 - 8 °C.
- Do not freeze and thaw the blood sample.
- For best conditions, the blood sample should be fresh, i.e. not more than six hours should have elapsed between sample collection and analysis.
- Blood samples can be used up to 24 hours after drawing if they are stored in the fridge at 2 – 8 °C.
- Before use, the sample tubes containing the blood must be inverted gently 8 to 10 times.
- Do not use clotted blood samples.

11 Examination procedure

NOTICE *Reverse pipetting is critical to accuracy especially when dispensing viscous and very small sample volumes. For whole blood pipetting, we recommend using a calibrated electronic pipette which is preprogrammed to operate in the reverse pipetting mode.*
If an electronic pipette is not available, follow these instructions for manual reverse pipetting:
- *Depress the operating button to the second stop. Let the operating button move up completely, excess sample is drawn up into the tip.*
- *Depress the operating button to the first stop to expel a precise volume of blood, leaving excess sample in the tip.*
- *Discard pipette tip with excess blood sample in the tip into medical waste container.*

Please note, you must not use reverse pipetting for antibody or buffer solutions.

11.1 Staining

- Invert the blood sample in the EDTA blood collection tube gently 8 to 10 times.
- Pipette 20 µl EDTA whole blood sample to the bottom of a sample tube, avoid leaving a trail of blood on the inner tube wall from pipette tip. Discard the pipette tip.
- Add 20 µl CD4 mAb PE directly into the blood sample and mix it gently, avoid leaving a trail of blood on the inner tube wall from pipette tip. Discard the pipette tip.
- The sample should not be vortexed at this stage of preparation.
- Incubate the mixture for 15 minutes at room temperature in the dark.
- Add 800 µl no lyse buffer and vortex briefly, or tap tube gently in order to mix the sample. Discard the pipette tip.
- Vortex briefly, or tab tube gently before analysing with the CyFlow™ Counter.

NOTICE *The stained blood sample must be analysed within 2 hours after adding the no lyse buffer.*

11.2 Sample analysis

Please refer to the IFU of the CyFlow™ Counter (CY-S-3022IFUEN) for how to operate the instrument before analysing the samples. The start-up procedures and the internal quality control procedure must be successfully completed before sample analysis.
- Choose and load the configuration for CD4 measurement from the ‚menu bar of CyFlow™ Counter.
- Insert the sample tube with the prepared blood sample into the sample port.
- Start measurement.
- After sample run, the instrument stops and cleans automatically.
- Remove and dispose of blood sample in accordance with local laboratory biohazard safety procedures.

Do not start a second measurement with the same sample tube. If a duplicate or repeat measurement is required, a second sample tube should be prepared as per 11.1.

11.3 Data acquisition and analysis

Data analysis with the CyFlow™ Counter is only possible if the internal quality control of the instrument was successful.
Please refer to the IFU of the CyFlow™ Counter (CY-S-3022IFUEN) for proper start-up procedure and maintenance of the device, if necessary.
The CD4 T-cell counting result will be displayed on the screen.
If the histogram does not show a clear peak of the CD4 positive stained T-cells, the sample preparation and measurement must be repeated.
Results will be incorrect if gate positioning is not done precisely.
Be careful not to gate monocytes as CD4 positive stained cells if CD4 concentration is extremely low in a patient blood sample.

12 Calculation of examination results

Calculation of the cell concentration is part of the software-based data analysis, which provides the result in CD4 cells per µl blood sample after each measurement.
For further information, please refer to the IFU of the CyFlow™ Counter (CY-S-3022IFUEN).

13 Interpretation of results

Human Immunodeficiency Virus (HIV) is one of the main reasons for CD4 cell depletion, which debilates the immune system. Results should be interpreted by physicians, in conjunction with the locally applicable HIV treatment guidelines.

14 Control procedure

Appropriate quality control should be performed according to local and national regulations. A variety of stabilized blood samples are available on the market, only a few are suitable when using certain flow cytometers.If the acceptance criterion of the individual control material is not met do not proceed with patient specimen testing and contact your local Sysmex representative.
For further information about the use of adequate control material, please contact your local Sysmex representative.

15 Performance characteristics

15.1 Specificity

The mouse monoclonal antibody MEM-241 recognizes the human CD4 antigen, a transmembrane glycoprotein (55 kDa) of the immunoglobulin supergene family, present on a subset of T-lymphocytes ("helper/inducer" T-cells) and expressed at a lower level on monocytes, tissue macrophages and granulocytes. HCDM (former HLDA VIII) Meeting, May 2006, Québec, Canada; WS Code M241 Detection capability. The evaluation for Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) was carried out according to the specifications in the guideline CLSI EP17-A2.
LoB: ≤50% of LoD
LoD: ≤20 CD4 cells/µl
LoQ: ≤40 CD4 cells/µl

15.2 Repeatability

Intra- and inter-assay variation:
CV values are less than 10% for CD4 values of 200 CD4 T-cells/µl or above.
CV values are less than 15% for CD4 absolute values below 200 CD4 T-cells/µl.

15.3 Linearity

The measuring range of the assay and linearity of the measurement are valid over the range:
CD4 absolute: 40-2500 CD4 T-cells/µl

15.4 Trueness

EDTA whole blood specimen were stained with CD4 easy count kit and analysed on the CyFlow™ Counter. CD4 absolute values were compared with results from a BD FACSCalibur using BD Tritest CD3/CD4/CD45 and BD Trucount tubes and showed a bias of -15,13% between both methods.

16 Limitations

CAUTION ⚠ *Sysmex Partec CD4 easy count kits (05-8401) have not been validated for use with pediatric and adolescent patients.*

CAUTION ⚠ *Patient measurements of CD4 and CD4% made using Sysmex Partec assays should not be used independently with other manufacturer's methods of determining CD4 and CD4%. Use the assays only with the Sysmex Partec instruments. Values obtained using other equipment or assays may not be interchangeable.*

Different endogenous and exogenous substances were tested regarding the CLSI EP07-A2 guideline for possible interfering effects which could have an influence to the blood sample analysis. Finally, ≥ 18% haemolysis (induced by using the protocol recommended in the CLSI EP07-A2, Appendix G, G1 - Osmotic Shock Procedure) was observed to show an interfering effect. For that reason haemolyzed samples should be rejected.
For further questions regarding the performance of the product, please contact your local Sysmex representative.

17 Literature references

1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondière, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
2) L. Zijenah, G. Kadzirange, S. Madzime, M. Borok, C. Mudiwa, O. Tobaiwa, M. Mucheche, S. Rusakaniko, D. Katzenstein. (2006). Affordable flow cytometry for enumeration of absolute CD4+ T-lymphocytes to identify subtype C HIV-I infected adults requiring antiretroviral therapy (ART) and monitoring response to ART in a resource-limited setting. Journal of Translational Medicine 4:33
3) H. Karcher, D. Bohning, R. Downing, S. Mashate, G. Harms. (2006). Comparison of two alternative methods for CD4+ T-cell determination (Coulter manual CD4 count and CyFlow) against standard dual platform flow cytometry in Uganda. Clinical Cytometry, 70B (3):163-69
4) S. Kohreanudom, S. Dettraira. (2012). Evaluation of the Partec Single-Platform Volumetric CyFlow® Counter System for Determining Percentage and Absolute Numbers of CD4 T Lymphocytes in HIV/AIDS Thai Patients. Thai AIDS J, 25:1-10
5) D. Wade, P.A. Diaw, G. Daneau, M. Camara, T.N. Dieye, S. Mboup, L. Kestens. (2013). CD4 T-Cell Enumeration in a Field Setting: Evaluation of CyFlow Counter Using the CD4 Easy Count Kit-Dry and Pima CD4 Systems. PLoS ONE, 8(9): e75484

18 Contact of manufacturer

Manufacturer
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Germany
www.sysmex-partec.com
Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Version number of IFU and date of issue

Revision: Rev-007_10-09-2020_CN 1563
Issued by: Sysmex Partec GmbH

Safety Data Sheet for this product is available at www.sysmex-partec.com/services.

Benutzerhandbuch

Identifikation des IVD-Reagens

Produktname:

CD4 easy count kit

REF 05-8401

Inhalt:

Ampulle mit CD4 mAb PE — REF 05-8401-01
Flasche mit No-Lyse-Puffer — REF 05-8401-02

1 Spezifikation

Spezifität	Humanes CD4
Isotyp	IgG1
Inhalt	100 Tests
Fluorochrom	PE
λ Anregung (nm)	532 / 488
Emissionsmaximum (nm)	578

2 Zweckbestimmung

IVD In-vitro-Diagnostikum.

Das CD4 easy count kit ist ein manueller, quantitativer 2-Komponenten-In-vitro-Test für die Markierung von Lymphozyten-Untergruppen in venösen EDTA-Vollblutproben von Erwachsenen und deren anschließender Auszählung mit dem Sysmex Partec IVD-Flowzytometer CyFlow™ Counter. Die Konzentration der CD4-positiven T-Helferzellen in Blutproben ist in Verbindung mit anderen Laborwerten und klinischen Befunden ein nützlicher Indikator für die Einleitung der Behandlung HIVpositiver Patienten und die Nachsorge.
Der Test ist nur von geschulten medizinischen Fachkräften durchzuführen.

3 Prinzip des Untersuchungsverfahrens

Ein Aliquot einer EDTA-Vollblutprobe wird mit dem an das Fluorochrom konjugierten Antikörper (CD4) in einem Verhältnis von 1:1 gemischt, Nach einer bestimmten Inkubationszeit wird der Puffer hinzugegeben; nun kann die Analyse der Probe in einem CyFlow™ Counter-Flowzytometer erfolgen. Die Lichtquelle regt den Fluoreszenzfarbstoff an, mit dem die Zellen markiert wurden, und das emittierte Licht wird, während ein bestimmtes Volumen der Blutprobe das Instrument durchläuft, detektiert. Die Konzentration der spezifischen Zellpopulation wird von der integrierten Software berechnet. Weitere Informationen entnehmen Sie bitte dem Benutzerhandbuch des CyFlow™ Counter (CY-S-3022IFUEN).

4 Lagerung und Haltbarkeitsdauer nach dem ersten Öffnen

1. Lagerung:
Das Antikörper- und Pufferreagens bei 2 – 8 °C im Dunkeln aufbewahren. Nicht einfrieren oder erhöhten Temperaturen aussetzen. Vor direkter Sonneneinstrahlung schützen.
2. Haltbarkeitsdauer nach dem ersten Öffnen:
Die Flasche nach jedem Gebrauch wieder verschließen und für jede weitere Entnahme des Reagens neue Pipettenspitzen verwenden, um eine Verunreinigung zu vermeiden.
Das Antikörper- und Pufferreagens bei 2 – 8 °C im Dunkeln aufbewahren. Nicht einfrieren oder erhöhten Temperaturen aussetzen. Vor direkter Sonneneinstrahlung schützen.
Unter Einhaltung der oben genannten Lagerbedingungen ist das CD4 easy count kit bis zum Verfallsdatum auf dem Etikett des Kits stabil.

5 Bestandteile

CD4 mAb PE ist ein muriner monoklonaler Antikörper in PBS-Puffer mit 0,2 % BSA und 0,09 % Natriumazid.
No-Lyse-Puffer ist eine Lösung auf PBS-Basis, die 0,09 % Natriumazid enthält.

6 Anzeichen von Verderb

Die Antikörper- und Pufferlösungen sind klare Flüssigkeiten. Die Reagenzien nicht verwenden, falls Trübungen oder Verunreinigungen auftreten.
Bei Fragen zur Leistung oder Qualität des Produkts wenden Sie sich bitte an Ihre nächstgelegene Sysmex-Vertretung.

7 Vorsichtsmaßnahmen und Warnhinweise

Die Reagenzien enthalten 14 mM Natriumazid als Konservierungsstoff. Die geringe Konzentration von Natriumazid erfordert keine Gefahrenkennzeichnung, jedoch sind die beim Umgang mit Chemikalien üblichen Vorsichtsmaßregeln zu beachten. Beachten Sie das Sicherheitsdatenblatt (SDB) für eine vollständige Liste von Warnhinweise und Vorsichtsmaßnahmen.

8 Zusätzlich erforderliche Ausrüstung

Erforderliche Instrumente:	CyFlow™ Counter (REF No.: CY-S-3022)
Benötigte Ausrüstung:	Sample Tubes 3.5 ml (REF No.: 04-2000) Eine verifizierte 20-µl-Pipette und Pipettenspitzen Eine verifizierte 100- bis 1000-µl-Pipette und Pipettenspitzen System zur venösen Blutentnahme mit EDTA als Antikoagulans Stoppuhr

9 Reagensvorbereitung

CD4 mAb PE (REF No.: 05-8401-01), das Antikörperreagens ist gebrauchsfertig.
No-Lyse-Puffer (REF No.: 05-8401-02), die Pufferlösung ist gebrauchsfertig.

10 Entnahme, Handhabung und Lagerung der Primärprobe

WARNUNG ⚠ *Alle biologischen Proben und Materialien sind als gefährliches biologisches Material und als potenziell infektiös zu behandeln. Es sind angemessene Sicherheitsvorkehrungen und Verfahren zur Handhabung gemäß den geltenden Gesetzen und Vorschriften anzuwenden.*

- Bei der Blutprobe muss es sich um eine venöse Vollblut-Probe handeln, die mithilfe eines Blutentnahmesystems entnommen wurde, das EDTA als Antikoagulans verwendet.
- Die Blutprobe ist in einem dunklen, vor Licht geschützten Behälter zu transportieren und darf nicht erhöhten Temperaturen ausgesetzt werden. Ein geeigneter Transportbehälter ist z. B. eine Styroporbox mit Kühlung. Die besten Transportbedingungen würden unter 25 °C liegen, wenn der Transport nicht länger als 6 Stunden nach der Spende dauern würde. Für eine Transport- und Lagerzeit von mehr als 6 Stunden muss das Blut bei 2 - 8 °C aufbewahrt werden.
- Die Blutprobe nicht einfrieren und wieder auftauen.
- Optimale Bedingungen sind bei Verwendung einer frischen Blutprobe gegeben, d. h. es sollten nicht mehr als sechs Stunden zwischen der Blutentnahme und der Analyse liegen.
- Bei Lagerung im Kühlschrank bei 2–8 °C können Blutproben bis zu 24 Stunden nach der Entnahme verwendet werden.
- Vor der Anwendung die Probenröhrchen 8- bis 10-mal vorsichtig umdrehen.
- Keine geronnenen Blutproben verwenden.

11 Untersuchungsverfahren

HINWEIS *Reverses Pipettieren ist entscheidend für die Genauigkeit, insbesondere bei der Abgabe viskoser und sehr kleiner Probenmengen. Für das Pipettieren von Vollblut empfehlen wir die Verwendung einer kalibrierten elektronischen Pipette, die für den Betrieb im reversen Pipettiermodus vorprogrammiert ist.*
Steht keine elektronische Pipette zur Verfügung, führen Sie das reverse Pipettieren gemäß den folgenden Anweisungen manuell durch:
- *Bedienknopf bis zum zweiten Anschlag herunterdrücken. Bedienknopf vollständig zurückgleiten lassen. Es wird eine größere Menge Probe in die Spitze aufgezogen.*
- *Bedienknopf bis zum ersten Anschlag herunterdrücken, um ein genaues Blutvolumen abzugeben. Anschließend befindet sich noch ein Rest der Probe in der Spitze.*
- *Pipettenspitze mit dem verbliebenen Rest der Blutprobe in einen Behälter für medizinische Abfälle entsorgen.*

Beachten Sie bitte, dass reverses Pipettieren nicht für Antikörper- oder Pufferlösungen eingesetzt werden darf.

11.1 Markierung

- Die Blutprobe im EDTA-Blutentnahmeröhrchen 8- bis 10-mal vorsichtig umdrehen.
- 20 µl EDTA-Vollblutprobe auf den Boden eines Probenröhrchens pipettieren. Dabei keine Blutspuren von der Pipettenspitze auf der Röhrcheninnenwand hinterlassen. Entsorgen Sie die Pipettenspitze.
- 20 µl CD4 mAb PE direkt in die Blutprobe geben und vorsichtig mischen. Dabei keine Blutspuren von der Pipettenspitze auf der Röhrcheninnenwand hinterlassen. Entsorgen Sie die Pipettenspitze.
- Probe zu diesem Zeitpunkt nicht vortexen.
- Inkubation für 15 Minuten bei 15-30 °C im Dunkeln.
- 800 µl No-Lyse-Puffer hinzugeben und kurz vortexen oder das Röhrchen sanft aufklopfen, um die Probe zu mischen. Entsorgen Sie die Pipettenspitze.
- Vor der Analyse mit dem CyFlow™ Counter kurz vortexen oder das Röhrchen sanft aufklopfen.

HINWEIS *Nach der Zugabe von No-Lyse-Puffer müssen die Proben innerhalb von 2 Stunden analysiert werden.*

11.2 Probenanalyse

Bitte vor der Probenanalyse das Benutzerhandbuch des CyFlow™ Counter (CY-S-3022IFUEN) für die Verwendung des Geräts beachten. Vor der Probenanalyse müssen die Verfahren zur Inbetriebnahme und internen Qualitätskontrolle erfolgreich abgeschlossen worden sein.
- Wählen und laden Sie die Konfiguration für die CD4-Messung aus der Menüleiste des CyFlow™ Counter.
- Das Probenröhrchen mit dem aufbereiteten Blut in den Probenport setzen.
- Die Messung starten.
- Nach dem Probendurchlauf stoppt das Instrument und wird automatisch gereinigt.
- Die Blutprobe entnehmen und gemäß den örtlichen Sicherheitsvorschriften in Bezug auf biogefährliche Materialien in Labors entsorgen.

Keine erneute Messung mit demselben Probenröhrchen durchführen. Wenn eine Zweifachmessung oder eine Wiederholung der Messung erforderlich ist, muss ein zweites Probenröhrchen wie unter 11.1 beschrieben vorbereitet werden.

11.3 Datenerfassung und -analyse

Die Datenanalyse kann erst erfolgen, wenn die interne Qualitätskontrolle des CyFlow™ Counter erfolgreich abgeschlossen wurde.
Im Benutzerhandbuch des CyFlow™ Counter (CY-S-3022IFUEN) finden Sie eine Beschreibung der korrekten Inbetriebnahme und Wartung des Geräts.
Die CD4-T-Zellzahl wird auf dem Bildschirm angezeigt. Zeigt das Histogramm keine eindeutige Erhöhung der markierten CD4-positiven T-Zellen an, muss die Probenvorbereitung und -messung wiederholt werden.
Es kommt zu falschen Ergebnissen, wenn das Gaten nicht korrekt durchgeführt wird.
Achten Sie darauf, Monozyten nicht als markierte CD4-positive Zellen zu gaten, wenn die CD4-Konzentration der Patientenblutprobe sehr niedrig ist.

12 Berechnung der Untersuchungsergebnisse

Die Berechnung der Zellkonzentration erfolgt im Rahmen der Software-basierten Datenanalyse. Die Ergebnisse werden nach jeder Messung als CD4-Zellen pro µl Blutprobe dargestellt.
Weitere Informationen entnehmen Sie bitte dem Benutzerhandbuch des CyFlow™ Counter (CY-S-3022IFUEN).

13 Interpretation der Ergebnisse

Das Humane Immundefizienzvirus (HIV) ist einer der Hauptgründe für die CD4-Zelldepletion, die das Immunsystem beeinträchtigt. Die Ergebnisse sollten von Ärzten in Verbindung mit lokal anwendbaren HIV-Behandlungsrichtlinien interpretiert werden.

14 Kontrollverfahren

Es sollte eine geeignete Qualitätskontrolle gemäß den geltenden nationalen Laborvorschriften durchgeführt werden.
Im Handel ist ein breites Angebot an stabilisierten Blutproben erhältlich, jedoch sind bei Verwendung bestimmter Flowzytometer nur wenige geeignet.
Wenn das Annahmekriterium des betreffenden Kontrollmaterials nicht erfüllt ist, die Patientenprobe nicht analysieren. Wenden Sie sich in diesem Fall an Ihre nächstgelegene Sysmex-Vertretung.
Weitere Informationen über die Verwendung geeigneten Kontrollmaterials können Sie ebenfalls bei Ihrer nächstgelegenen Sysmex-Vertretung erhalten.

15 Leistungsmerkmale

15.1 Spezifität

Der monoklonale Maus-Antikörper MEM-241 erkennt das humane CD4-Antigen, ein transmembranes Glykoprotein (55 kDa) der Immunglobulin-Superfamilie, das auf einer Untergruppe von T-Lymphozyten (T-Helferzellen) exprimiert wird, sowie in geringerem Maße auf Monozyten, Gewebsmakrophagen und Granulozyten. HCDM (ehemals HLDA VIII) Meeting, Mai 2006, Québec, Kanada; WS Code M241

15.2 Nachweisfähigkeit

Die Leerwert-Obergrenze (Limit of Blank, LoB), die Nachweisgrenze (Limit of Detection, LoD) und die Quantifizierungsgrenze (Limit of Quantitation, LoQ) wurden gemäß den Spezifikationen der Richtlinie CLSI EP17-A2 ermittelt.
LoB: ≤ 50 % der LoD
LoD: ≤ 20 CD4-Zellen/µl
LoQ: ≤ 40 CD4-Zellen/µl

15.3 Reproduzierbarkeit

Intra- und Inter-Assay-Varianz:
Die CV-Werte für CD4-Werte ≥ 200 CD4-T-Zellen/µl liegen unter 10 %.
Die CV-Werte für absolute CD4-Werte < 200 CD4-T-Zellen/µl liegen unter 15 %.

15.4 Linearität

Der Messbereich des Assays und die Linearität der Messungen gelten für den folgenden Bereich:
Absoluter CD4-Wert: 40–2500 CD4-T-Zellen/µl.

15.5 Richtigkeit

EDTA-Vollblutproben wurden mit dem CD4 easy count kit markiert und im CyFlow™ Counter analysiert. Die absoluten CD4-Werte wurden mit Ergebnissen eines BD FACSCalibur unter Verwendung von BD Tritest CD3/CD4/CD45 und BD Trucount-Röhrchen verglichen. Es zeigte sich eine Abweichung von -22,56 % zwischen den Methoden.

16 Grenzen des Verfahrens

VORSICHT ⚠ *Die Sysmex Partec CD4 easy count kits (05-8401) wurden nicht für die Verwendung bei Kindern und Jugendlichen validiert.*

VORSICHT ⚠ *Patientenmessungen von CD4 und CD4% mithilfe von Sysmex Partec Assays sollten nicht zusammen mit Methoden anderer Hersteller zur Bestimmung von CD4 und CD4% verwendet werden. Die Assays sollten nur mit den Sysmex Partec Geräten verwendet werden. Die Messwerte sind unter Umständen nicht beliebig austauschbar mit Werten, die mit anderen Geräten oder Assays gemessen wurden.*

Verschiedene endogene und exogene Substanzen wurden in Übereinstimmung mit der Richtlinie CLSI EP07-A2 auf eine mögliche Beeinträchtigung der Blutprobenanalyse untersucht.
Bei ≥ 18 % Hämolyse (gemäß dem in der Richtlinie CLSI EP07-A2, Anhang G empfohlenen Verfahren G1 „Osmotic Shock Procedure" induziert) wurde eine Beeinträchtigung beobachtet. Aus diesem Grund sollten hämolytische Proben verworfen werden.
Bei weiteren Fragen hinsichtlich der Leistung des Produkts wenden Sie sich bitte an Ihre nächstgelegene Sysmex-Vertretung.

17 Literatur

1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondière, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
2) L. Zijenah, G. Kadzirange, S. Madzime, M. Borok, C. Mudiwa, O. Tobaiwa, M. Mucheche, S. Rusakaniko, D. Katzenstein. (2006). Affordable flow cytometry for enumeration of absolute CD4+ T-lymphocytes to identify subtype C HIV-I infected adults requiring antiretroviral therapy (ART) and monitoring response to ART in a resource-limited setting. Journal of Translational Medicine 4:33
3) H. Karcher, D. Bohning, R. Downing, S. Mashate, G. Harms. (2006). Comparison of two alternative methods for CD4+ T-cell determination (Coulter manual CD4 count and CyFlow) against standard dual platform flow cytometry in Uganda. Clinical Cytometry, 70B (3):163-69
4) S. Kohreanudom, S. Dettraira. (2012). Evaluation of the Partec Single-Platform Volumetric CyFlow® Counter System for Determining Percentage and Absolute Numbers of CD4 T Lymphocytes in HIV/AIDS Thai Patients. Thai AIDS J, 25:1-10
5) D. Wade, P.A. Diaw, G. Daneau, M. Camara, T.N. Dieye, S. Mboup, L. Kestens. (2013). CD4 T-Cell Enumeration in a Field Setting: Evaluation of CyFlow Counter Using the CD4 Easy Count Kit-Dry and Pima CD4 Systems. PLoS ONE, 8(9): e75484

18 Kontaktinformationen des Herstellers

Hersteller
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Germany
www.sysmex-partec.com
Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Versionsnummer des Benutzerhandbuchs und Ausgabedatum

Revision: Rev-07_10-09-2020_CN 1563
Ausgegeben von: Sysmex Partec GmbH

Das Sicherheitsdatenblatt zu diesem Produkt ist unter www.sysmex-partec.com/services erhältlich.

Guide d'utilisation

Identification du réactif IVD

Nom du produit :

CD4 easy count kit

REF 05-8401

Contenu :

Fiole de CD4 mAb PE — REF 05-8401-01
Flacon de no lyse buffer (tampon sans lyse) — REF 05-8401-02

1 Spécification

Spécificité	CD4 humain
Isotype	IgG1
Contenu	100 tests
Fluorochrome	PE
Excitation λ (nm)	532 / 488
Émission maximale (nm)	578

2 Usage prévu

IVD Pour la réalisation de diagnostics in vitro

Le CD4 easy count kit est un test IVD quantitatif manuel constitué de deux composants et destiné au marquage des sous-populations de lymphocytes dans le sang veineux total EDTA chez l'adulte, puis à leur dénombrement à l'aide du cytomètre en flux IVD CyFlow™ Counter de Sysmex Partec.
La concentration en cellules T CD4 des échantillons de sang est un indicateur utile pour l'initiation ou le suivi du traitement des porteurs du VIH, conjointement avec d'autres résultats cliniques et de laboratoire.
Le test doit être effectué par des professionnels de santé formés à cet effet.

3 Principe de la méthode d'examen

Une aliquote d'un échantillon de sang total d'EDTA est mélangée à l'anticorps (CD4) conjugué au fluorochrome dans un rapport 1:1, Après un temps d'incubation déterminé, le tampon est ajouté et l'échantillon est prêt pour l'analyse sur un cytomètre en flux CyFlow™ Counter.
La source lumineuse excite la teinture fluorescente liée à la cellule marquée et la lumière émise est détectée pendant le passage d'un certain volume d'échantillon de sang dans l'instrument. Le logiciel intégré calcule la concentration de la population cellulaire spécifique.
Pour de plus amples informations, se reporter au guide d'utilisation du CyFlow™ Counter (CY-S-3022IFUEN).

4 Stockage et durée de conservation après la première ouverture

1. Stockage :
Stocker l'anticorps et les réactifs tampons à une température comprise entre 2 et 8 °C à l'abri de la lumière. Ne pas congeler ni exposer les réactifs à des températures élevées et les protéger du rayonnement solaire direct.
2. Durée de conservation après la première ouverture :
Toujours fermer le flacon après usage et utiliser de nouveaux embouts de pipettes à chaque fois qu'un échantillon de réactif est prélevé pour éviter toute contamination.
Conserver l'anticorps et le réactif tampon à une température comprise entre 2 et 8 °C à l'abri de la lumière. Ne pas congeler ni exposer les réactifs à des températures élevées et les protéger du rayonnement solaire direct.
Dans ces conditions de stockage, le CD4 easy count kit restera stable jusqu'à la date de péremption imprimée sur l'étiquette du kit.

5 Composants

Le CD4 mAb PE est un anticorps monoclonal murin fourni dans un tampon PBS avec 0,2 % de BSA et 0,09 % d'azoture de sodium. Le tampon sans lyse est une solution à base de PBS contenant 0,09 % d'azoture de sodium.

6 Signes de détérioration

La solution d'anticorps et la solution tampon sont des liquides clairs. Ne pas utiliser les réactifs en cas d'apparition d'un quelconque signe de turbidité ou de contamination.
Pour toutes questions concernant la performance ou la qualité du produit reçu, veuillez contacter votre représentant Sysmex local.

7 Avertissements et mesures de précaution

Les réactifs contiennent de l'azoture de sodium 14 mM comme agent conservateur. La faible concentration de l'azoture de sodium ne nécessite aucun marquage comme substance dangereuse, mais les mesures de sécurité normales relatives à la manipulation des produits chimiques doivent être observées. Se reporter à la fiche de données de sécurité pour obtenir la liste complète des avertissements et mesures de précaution.

8 Équipement supplémentaire requis

Instruments requis	CyFlow™ Counter (REF No.: CY-S-3022)
Matériel requis	Tubes d'échantillon (REF No.: 04-2000) Une pipette 20 µl validée et embouts de pipette Une pipette 100 – 1000 µl validée et embouts de pipette Système de prélèvement de sang veineux avec EDTA comme anticoagulant Chronomètre

9 Préparation des réactifs

CD4 mAb PE (05-8401-01), le réactif à base d'anticorps est prêt à l'emploi.
no lyse buffer (05-8401-02), la solution tampon est prête à l'emploi.

10 Prélèvement, manipulation et stockage de l'échantillon primaire

AVERTISSEMENT ⚠ *Tous les échantillons et matériaux biologiques sont considérés comme biologiquement dangereux et doivent être manipulés comme s'ils étaient susceptibles de transmettre une infection. Les mesures de sécurité et les procédures de manipulation appropriées doivent être appliquées conformément aux lois et aux réglementations en vigueur.*

- L'échantillon de sang doit être un échantillon de sang veineux total, prélevé à l'aide d'un système de prélèvement sanguin contenant de l'EDTA comme anticoagulant.
- L'échantillon de sang doit être transporté dans un récipient sombre, hermétique à la lumière, et ne doit pas être exposé à des températures élevées. Une boîte en polystyrène avec un dispositif de réfrigération, par exemple, constitue un conteneur de transport approprié. Les meilleures conditions de transport seraient inférieures à 25 °C si le transport ne prend pas plus de 6 heures après le don. Pour le transport et le stockage plus de 6 heures, le sang doit être conservé entre 2 et 8 °C.
- Ne pas congeler ni décongeler l'échantillon de sang.
- Pour des conditions optimales, l'échantillon de sang doit être frais, c'est-à-dire qu'il ne doit pas s'être écoulé plus de six heures entre le prélèvement de l'échantillon et l'analyse.
- Les échantillons de sang peuvent être utilisés jusqu'à 24 heures après la prise de sang, s'ils sont conservés au réfrigérateur à une température comprise entre 2 et 8 °C.
- Avant l'utilisation, les tubes d'échantillon de sang doivent être retournés doucement 8 à 10 fois.
- Ne pas utiliser des échantillons de sang coagulé.

11 Procédure d'examen

REMARQUE *Le pipettage à l'envers est essentiel à la précision, tout particulièrement en cas de délivrance d'échantillons visqueux et de volumes d'échantillons très réduits. Pour le pipettage de sang total, nous recommandons l'utilisation d'une pipette électronique calibrée qui est préprogrammée pour fonctionner en mode de pipettage à l'envers.*
En l'absence d'une pipette électronique, suivre les présentes consignes pour procéder à un pipettage manuel à l'envers :
- *Enfoncer le bouton d'actionnement jusqu'à la deuxième butée. Laisser le bouton remonter entièrement, l'excès d'échantillon est aspiré dans la pointe.*
- *Enfoncer le bouton d'actionnement jusqu'à la première butée pour expulser un volume précis de sang tout en conservant l'excès d'échantillon dans la pointe.*
- *Éliminer la pointe de pipette contenant l'excès d'échantillon de sang dans une boîte à déchets médicaux.*

Prière de noter qu'il n'est pas nécessaire de procéder au pipettage à l'envers pour les anticorps ou les solutions tampons.

11.1 Marquage

- Renverser doucement l'échantillon de sang dans le tube de prélèvement de sang EDTA 8 à 10 fois.
- Pipeter 20 µl d'échantillon de sang total EDTA dans le fond d'un tube d'échantillon, en évitant de laisser un filet de sang sur la paroi intérieure du tube avec l'embout de pipette. Jeter la pointe de la pipette.
- Ajouter 20 µl de CD4 mAb PE directement dans l'échantillon de sang et mélanger doucement en évitant de laisser un filet de sang sur la paroi intérieure du tube avec l'embout de pipette. Jeter la pointe de la pipette.
- Exemple à ce moment ne pas vortexer.
- Incuber le mélange pendant 15 minutes à 15-30 °C dans l'obscurité.
- Ajouter 800 µl de no lyse buffer (tampon sans lyse) et mélanger brièvement au vortex ou tapoter doucement le tube afin de mélanger l'échantillon. Jeter la pointe de la pipette.
- Mélanger brièvement au vortex ou tapoter doucement le tube avant d'effectuer l'analyse à l'aide du CyFlow™ Counter.

REMARQUE *Les échantillons doivent être analysés dans un délai de 2 heures après l'ajout de no lyse buffer (tampon sans lyse).*

11.2 Analyse de l'échantillon

Pour obtenir des informations sur le fonctionnement de l'instrument avant l'analyse des échantillons, se reporter au guide d'utilisation du CyFlow™ Counter (CY-S-3022IFUEN). Les procédures de démarrage et la procédure de contrôle qualité interne doivent avoir été effectuées avec succès avant l'analyse de l'échantillon.
- Choisissez et chargez la configuration pour la mesure CD4 à partir de la barre de menu du CyFlow™ Counter.
- Insérer le tube d'échantillon avec l'échantillon de sang préparé dans l'orifice d'échantillonnage.
- Démarrer la mesure.
- Après l'analyse de l'échantillon, l'instrument s'arrête et se nettoie automatiquement.
- Retirer et éliminer l'échantillon de sang conformément aux procédures locales de sécurité en laboratoire relatives aux risques biologiques.

Ne pas démarrer de seconde mesure avec le même tube d'échantillon. S'il faut répéter ou effectuer deux fois la mesure, préparer un second tube d'échantillon conformément au point 11.1.

11.3 Acquisition et analyse des données

L'analyse des données à l'aide du CyFlow™ Counter n'est possible que si le contrôle qualité interne de l'instrument a été effectué avec succès.
Consulter le guide d'utilisation du CyFlow™ Counter (CY-S-3022IFUEN) pour effectuer une procédure de démarrage correcte et la maintenance de l'appareil, si nécessaire.
Le résultat du comptage des cellules T CD4 s'affiche à l'écran.
Si l'histogramme n'indique pas un pic clair des cellules T marquées positives CD4, il faut recommencer la préparation et la mesure de l'échantillon.
Les résultats seront incorrects si le positionnement de la fenêtre électronique (gate) n'est pas réalisé avec précision.
Prendre garde à ne pas fenêtrer les monocytes en tant que cellules marquées positives CD4, si la concentration en CD4 dans l'échantillon de sang du patient est extrêmement faible.

12 Calcul des résultats d'examen

Le calcul de la concentration cellulaire fait partie intégrante de l'analyse des données par logiciel qui fournit le résultat en cellules CD4 par µl d'échantillon de sang, après chaque mesure.
Pour de plus amples informations, se reporter au guide d'utilisation du CyFlow™ Counter (CY-S-3022IFUEN).

13 Interprétation des résultats

Le virus de l'immu-nodéficience hu-maine (VIH) est l'une des princi-pales raisons de la déplétion des cellules CD4, qui affai-blit le système im-munitaire. Les ré-sultats doivent être interprétés par les médecins, en liai-son avec les directives de traitement du VIH applicables localement.

14 Procédure de contrôle

Un contrôle qualité approprié doit être effectué conformément aux réglementations des laboratoires nationaux.
Il est recommandé d'utiliser un échantillon de sang de contrôle (05-8993 Control Blood-dry), avec une quantité spécifique de cellules CD4 pour le contrôle qualité quotidien de la préparation de l'échantillon et du comptage de l'instrument.

15 Caractéristiques de performance

15.1 Spécificité

L'anticorps monoclonal de souris MEM-241 reconnaît l'antigène CD4 humain, une glycoprotéine transmembranaire (55 kDa) de la superfamille des immunoglobulines présente sur une souspopulation de lymphocytes T (cellules T « auxiliaires/inductrices ») et exprimée à un niveau moindre sur les monocytes, les macrophages tissulaires et les granulocytes. HCDM (former HLDA VIII) Meeting, mai 2006, Québec, Canada ; WS Code M241

15.2 Capacité de détection

Les évaluations de la limite du blanc (LoB), de la limite de détection (LoD) et de la limite de quantification (LoQ) ont été réalisées conformément aux spécifications de la directive CLSI EP17-A2.
LoB : ≤ 50 % de la LoD
LoD : ≤ 20 cellules CD4/µl
LoQ : ≤ 40 cellules CD4/µl

15.3 Répétabilité

Variation intra- et inter-essai :
Les valeurs du coefficient de variation (CV) sont inférieures à 10 % pour des valeurs de CD4 supérieures ou égales à 200 cellules T CD4/µl.
Les valeurs du coefficient de variation (CV) sont inférieures à 15 % pour des valeurs absolues de CD4 inférieures à 200 cellules T CD4/µl.

15.4 Linéarité

La plage de mesure de l'essai et la linéarité de la mesure sont valides sur la plage :
taux absolu de CD4 : 40–2500 cellules T CD4/µl.

15.5 Justesse

Les échantillons de sang total EDTA ont été marqués avec le CD4 easy count kit et analysés sur le CyFlow™ Counter. Les valeurs absolues de CD4 ont été comparées aux résultats obtenus à l'aide d'un BD FACSCalibur en utilisant des réactifs BD Tritest CD3/CD4/CD45 et des tubes BD Trucount, ce qui a révélé un écart de -22,56 % entre les deux méthodes.

16 Limites de la procédure

PRUDENCE ⚠ *L'utilisation des kits CD4 easy count (05-8401) Sysmex Partec n'a pas été validée chez les patients pédiatriques et adolescents.*

PRUDENCE ⚠ *Les mesures du taux de CD4 et du pourcentage CD4% réalisées chez des patients à l'aide de tests Sysmex Partec ne doivent pas être utilisées de manière indépendante avec d'autres méthodes de fabricant visant à la détermination du taux de CD4 et du pourcentage CD4%. N'utiliser les tests qu'avec les instruments Sysmex Partec. Les valeurs obtenues à l'aide d'autres équipements ou tests sont susceptibles de ne pas être interchangeables.*

Différentes substances endogènes et exogènes ont été testées conformément à la directive CLSI EP07-A2 pour vérifier les éventuelles interférences pouvant avoir une influence sur l'analyse de l'échantillon de sang. Il a finalement été observé ≥ 18 % d'hémolyse (engendrée par l'utilisation du protocole recommandé dans la directive CLSI EP07-A2, annexe G, G1 – Procédure de choc osmotique) provoquant des interférences. C'est pourquoi les échantillons hémolysés devrait être écartés.
Pour toutes questions supplémentaires concernant la performance du produit, veuillez contacter votre représentant Sysmex local.

17 Bibliographie

1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondière, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
2) L. Zijenah, G. Kadzirange, S. Madzime, M. Borok, C. Mudiwa, O. Tobaiwa, M. Mucheche, S. Rusakaniko, D. Katzenstein. (2006). Affordable flow cytometry for enumeration of absolute CD4+ T-lymphocytes to identify subtype C HIV-I infected adults requiring antiretroviral therapy (ART) and monitoring response to ART in a resource-limited setting. Journal of Translational Medicine 4:33
3) H. Karcher, D. Bohning, R. Downing, S. Mashate, G. Harms. (2006). Comparison of two alternative methods for CD4+ T-cell determination (Coulter manual CD4 count and CyFlow) against standard dual platform flow cytometry in Uganda. Clinical Cytometry, 70B (3):163-69
4) S. Kohreanudom, S. Dettraira. (2012). Evaluation of the Partec Single-Platform Volumetric CyFlow® Counter System for Determining Percentage and Absolute Numbers of CD4 T Lymphocytes in HIV/AIDS Thai Patients. Thai AIDS J, 25:1-10
5) D. Wade, P.A. Diaw, G. Daneau, M. Camara, T.N. Dieye, S. Mboup, L. Kestens. (2013). CD4 T-Cell Enumeration in a Field Setting: Evaluation of CyFlow Counter Using the CD4 Easy Count Kit-Dry and Pima CD4 Systems. PLoS ONE, 8(9): e75484

18 Coordonnées du fabricant

Fabricant
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Germany
www.sysmex-partec.com
Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Numéro de version du guide d'utilisation et date de publication

Révision : Rev-07_10-09-2020_CN 1563
Publiée par : Sysmex Partec GmbH

La fiche de données de sécurité de ce produit est disponible à l'adresse suivante : www.sysmex-partec.com/services.

IVD CE

Instrucciones de uso

Identificación del reactivo para DIV

Nombre del producto:

Kit CD4 easy count

REF 05-8401

Contenido:

Vial con mAb CD4-PE — REF 05-8401-01
Botella con solución amortiguadora sin lisis — REF 05-8401-02

1 Descripción

Especificidad	CD4 humano
Isotipo	IgG1
Contenido	100 tests
Fluorocromo	PE
λ de excitación (nm)	532 / 488
Emisión máxima (nm)	578

2 Uso previsto

IVD Para diagnóstico in vitro.
El kit CD4 easy count es un test cuantitativo manual de DIV de dos componentes para el marcaje de subpoblaciones de linfocitos en sangre entera venosa adulta con EDTA y la subsiguiente enumeración con el citómetro de flujo para DIV CyFlow™ Counter de Sysmex Partec. La concentración de linfocitos T CD4 de las muestras de sangre es un indicador útil para iniciar o supervisar el tratamiento de personas infectadas por el VIH junto con otros hallazgos clínicos y de laboratorio.
El test está previsto para su uso por parte de profesionales sanitarios con la formación correspondiente.

3 Principio del método de análisis

Se mezcla una alícuota de una muestra de EDTA de sangre completa con el anticuerpo (CD4) conjugado con el fluorocromo en una proporción 1:1, Tras un tiempo fijo de incubación, se añade la solución amortiguadora y la muestra está lista para su análisis en un citómetro de flujo CyFlow™ Counter.
La fuente de luz excita el colorante fluorescente unido a la célula teñida; se detecta la luz emitida durante el paso de un determinado volumen de sangre a través del instrumento. La concentración de la población celular específica se calcula con el software integrado.
Para más información, consulte las instrucciones de uso de CyFlow™ Counter (CY-S-3022IFUEN).

4 Almacenamiento y tiempo de conservación tras abrir por primera vez

1. Almacenamiento:
Almacene los reactivos de anticuerpos y solución amortiguadora en un lugar oscuro a 2-8 °C. No congele ni exponga los reactivos a temperaturas elevadas o a la luz solar directa.
2. Tiempo de conservación tras abrir por primera vez:
Cierre siempre la botella después de su uso y utilice puntas de pipeta nuevas cada vez que tome muestras del reactivo para evitar la contaminación.
Mantenga el reactivo de anticuerpos y solución amortiguadora en un lugar oscuro a 2-8 °C. No congele ni exponga los reactivos a temperaturas elevadas o a la luz solar directa.
En estas condiciones de almacenamiento, el kit CD4 easy count permanecerá estable hasta la fecha de caducidad impresa en la etiqueta del kit.

5 Componentes

mAb CD4-PE es un anticuerpo monoclonal murino suministrado en una solución amortiguadora PBS con un 0,2% de SAB y un 0,09% de azida de sodio. La solución amortiguadora sin lisis es una solución con base de PBS que contiene un 0,09% de azida de sodio.

6 Indicios de deterioro

El reactivo de anticuerpos y la solución amortiguadora son líquidos transparentes. No utilice el reactivo si aparece cualquier tipo de turbidez o contaminación.
Si tiene alguna pregunta con respecto al funcionamiento o la calidad del producto recibido, contacte con su representante local de Sysmex.

7 Precaución y advertencias

Los reactivos contienen 14 mM de azida de sodio como conservante. Dada su reducida concentración de azida de sodio, no requiere un etiquetado como producto de riesgo, pero se deben observar las precauciones de seguridad normales para la manipulación de sustancias químicas. Consulte la ficha técnica de seguridad para obtener una lista completa de advertencias y precauciones.

8 Equipamiento adicional necesario

Instrumental necesario:	CyFlow™ Counter (REF No.: CY-S-3022)
Equipamiento requerido:	Tubos de ensayo de 3,5 ml (REF No.: 04-2000) Una pipeta verificada de 20 µl y puntas de pipeta Una pipeta verificada de 100-1000 µl y puntas de pipeta Sistema de extracción de sangre venosa con EDTA como anticoagulante Cronómetro

9 Preparación del reactivo

mAb CD4-PE (REF No.: 05-8401-01), el reactivo de anticuerpos está listo para su uso.
Solución amortiguadora sin lisis (REF No.: 05-8401-02), la solución amortiguadora está lista para su uso.

10 Principios básicos de extracción, manipulación y almacenamiento de muestras

ADVERTENCIA ⚠ *Todas las muestras y materiales biológicos se consideran material de riesgo biológico y deberían manipularse como si fueran potencialmente infecciosos. Se deben observar las precauciones de seguridad y procedimientos de manipulación adecuados conforme a la legislación y normativa.*

- La muestra de sangre debe ser una muestra de sangre entera venosa, extraída con un sistema de extracción de sangre que contenga EDTA como anticoagulante.
- La muestra de sangre debe transportarse en un contenedor oscuro, protegido de la luz y no expuesto a temperaturas elevadas. Un contenedor de transporte adecuado es, por ejemplo, una caja de poliestireno extruido con material refrigerante. Las mejores condiciones de transporte estarían por debajo de los 25 °C si el transporte no demorará más de 6 horas después de la donación. Para el transporte y el tiempo de almacenamiento de más de 6 horas, la sangre debe mantenerse a 2 - 8 °C.
- No se puede congelar y descongelar la muestra de sangre.
- En condiciones óptimas la muestra de sangre debería ser reciente, es decir, no deberían haber pasado más de seis horas entre la extracción y el análisis de la muestra.
- Las muestras de sangre se pueden utilizar hasta 24 horas después de la extracción si se almacenan en el frigorífico a 2-8 °C.
- Los tubos con las muestras de sangre se deben voltear con suavidad de 8 a 10 veces antes de ser utilizados.
- No utilice muestras de sangre coagulada.

11 Procedimiento de análisis

AVISO *El pipeteado inverso es fundamental para la precisión, en particular cuando se dispensan volúmenes de muestra viscosos y muy pequeños. Para el pipeteado de sangre completa, recomendamos emplear una pipeta electrónica calibrada que esté preprogramada para funcionar en modo de pipeteado inverso.*
Si no hay disponible una pipeta electrónica, siga las presentes instrucciones para llevar a cabo un pipeteado inverso manual:
- *Presione el botón de funcionamiento hasta el segundo tope. Deje que el botón de funcionamiento ascienda por completo; la muestra sobrante se aspirará a la punta.*
- *Presione el botón de funcionamiento hasta el primer tope para expulsar un volumen concreto de sangre, dejando así la muestra sobrante dentro de la punta.*
- *Elimine la punta de la pipeta que contiene la muestra de sangre sobrante en un contenedor para residuos médicos.*

Tenga en cuenta que no debe utilizar el pipeteado inverso para soluciones de anticuerpos ni soluciones tampón.

11.1 Tinción

- Voltee con suavidad de 8 a 10 veces el tubo de extracción de sangre con EDTA que contiene la muestra de sangre.
- Pase con la pipeta 20 µl de la muestra de sangre entera con EDTA al fondo de un tubo de muestra y evite manchar la pared interior del tubo con la sangre de la punta de la pipeta. Deseche la punta de la pipeta.
- Añada 20 µl de mAb CD4-PE directamente en la muestra de sangre y mézclelo suavemente, evitando manchar la pared interior del tubo con la sangre de la punta de la pipeta. Deseche la punta de la pipeta.
- Muestra en este momento no lo hacen vórtice.
- Incubar la mezcla durante 15 minutos a 15-30 °C en la oscuridad.
- Añada 800 µl de la solución amortiguadora sin lisis y agite brevemente con la agitadora vorticial, o bien golpee suavemente el tubo para mezclar la muestra. Deseche la punta de la pipeta.
- Agite brevemente mediante agitadora vorticial, o bien golpee suavemente el tubo antes del análisis con el CyFlow™ Counter.

AVISO *Las muestras se deben analizar en un periodo máximo de 2 horas tras la adición de la solución amortiguadora sin lisis.*

11.2 Análisis de la muestra

En las instrucciones de uso de CyFlow™ Counter (CY-S-3022IFUEN) puede consultar el modo del empleo del equipo antes de analizar las muestras. Los procedimientos de puesta en marcha y el proceso de control de calidad interno deben completarse con éxito antes de iniciar el análisis de la muestra.
- Elija y cargue la configuración para la medición de CD4 desde la barra de menú del CyFlow™ Counter.
- Inserte el tubo de muestras con la muestra de sangre preparada en el acoplamiento para muestras.
- Inicie la medición.
- Tras el análisis de la muestra, el dispositivo se detiene y se limpia automáticamente.
- Retire y deseche la muestra de sangre conforme a los procedimientos locales de seguridad en laboratorio frente a riesgos biológicos.

No inicie una segunda medición con el mismo tubo de muestras. Si se requiere una repetición de la medición, se debe preparar un segundo tubo de muestras conforme a 11.1.

11.3 Adquisición y análisis de datos

El análisis de datos con CyFlow™ Counter solo es posible si el control de calidad interno del instrumento ha resultado satisfactorio.
Consulte las instrucciones de uso del CyFlow™ Counter (CY-S-3022IFUEN) para realizar, si fuera necesario, el procedimiento de inicio y mantenimiento del dispositivo de forma adecuada. El resultado del recuento de las células T CD4 aparecerá en la pantalla. Si el histograma no muestra un pico claro de las células T con tinción positiva para CD4, se deben repetir la preparación de la muestra y la medición. Los resultados serán incorrectos si no se realiza una acotación precisa. Asegúrese de no acotar monocitos como células con tinción positiva para CD4 si la concentración de CD4 es extremadamente baja en la muestra de sangre de un paciente.

12 Cálculo de los resultados del análisis

El cálculo de la concentración celular forma parte del análisis de datos informático, el cual suministra el resultado en células CD4 por µl de muestra de sangre después de cada medición.
Para más información, consulte las instrucciones de uso de CyFlow™ Counter (CY-S-3022IFUEN).

13 Interpretación de los resultados

El virus de la inmu-nodeficiencia hu-mana (VIH) es uno de los principales motivos del ago-tamiento de las células CD4, que debilita el sistema inmunitario. Las respuestas deben ser interpretadas por los médicos, en conjunción con las pautas de trata-miento del VIH aplicables local-mente.

14 Procedimiento de control

Se debe realizar un control de calidad apropiado conforme a las normativas nacionales para laboratorios.
Se han comercializado diversas muestras de sangre estabilizada, pero solo unas pocas son adecuadas cuando se utilizan determinados citómetros de flujo.
Si no se cumple el criterio de aceptación del material de control específico, no prosiga con el análisis de la muestra del paciente y póngase en contacto con su representante local de Sysmex.
Para más información sobre el uso de material de control adecuado, póngase en contacto con su representante local de Sysmex.

15 Eficacia diagnóstica

15.1 Especificidad

El anticuerpo monoclonal murino MEM-241 reconoce el antígeno humano CD4, una glucoproteína transmembranaria (55 kDa) de la superfamilia de las inmunoglobulinas, presente en un subconjunto de linfocitos T (células T cooperadoras/inductoras) y expresada en menor medida en monocitos, macrófagos y granulocitos. Congreso de HCDM (anteriormente HLDA VIII), mayo de 2006, Quebec, Canadá. Código WS: M241.

15.2 Capacidad de detección

La evaluación del límite de inclusión (LI), el límite de detección (LD) y el límite de cuantificación (LC) se realizó de acuerdo con las indicaciones de la directriz CLSI EP17-A2.
LI: ≤50% del LD
LD: ≤20 células CD4/µl
LC: ≤40 células CD4/µl

15.3 Repetibilidad

Variación intra e interanalítica:
Los valores de volumen celular suponen menos del 10% para valores de CD4 ≥ 200 células T CD4/µl.
Los valores de volumen celular suponen menos del 15% para valores absolutos de CD4 inferiores a 200 células T CD4/µl.

15.4 Linealidad

El intervalo de medición del ensayo y la linealidad de la medición son válidos en el intervalo:
CD4 absoluto: 40–2500 células T CD4/µl.

15.5 Veracidad

Las muestras de sangre entera con EDTA se tiñeron con el kit CD4 easy count y se analizaron en el CyFlow™ Counter. Se compararon los valores de CD4 absoluto con los resultados de un BD FACSCalibur utilizando BD Tritest CD3/CD4/CD45 y tubos BD Trucount y se puso de manifiesto una divergencia del -22,56% entre ambos métodos.

16 Limitaciones

CUIDADO ⚠ *El uso de los kits de Sysmex Partec CD4 easy count (05-8401) no está validado para pacientes pediátricos y adolescentes.*

CUIDADO ⚠ *Las mediciones en pacientes de CD4 y CD4% mediante las pruebas de Sysmex Partec no deben usarse independientemente para determinar CD4 y CD4% con métodos de otros proveedores. Utilizar las pruebas exclusivamente con los instrumentos de Sysmex Partec. Es posible que los valores obtenidos mediante otros equipos o pruebas no sean intercambiables.*

Se analizaron diferentes sustancias endógenas y exógenas conforme a la directriz CLSI EP07-A2 para detectar posibles efectos de interferencia que pudieran influir en el análisis de la muestra de sangre. Finalmente, se observó un ≥18% de hemólisis (provocada por el uso del protocolo recomendado en CLSI EP07-A2, apéndice G, G1, «Procedimiento de choque osmótico») como muestra de un efecto de interferencia. Por ese motivo, se deben rechazar las muestras hemolizadas.
Si tiene alguna pregunta más con respecto al funcionamiento del producto, póngase en contacto con su representante local de Sysmex.

17 Referencias bibliográficas

1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondière, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
2) L. Zijenah, G. Kadzirange, S. Madzime, M. Borok, C. Mudiwa, O. Tobaiwa, M. Mucheche, S. Rusakaniko, D. Katzenstein. (2006). Affordable flow cytometry for enumeration of absolute CD4+ T-lymphocytes to identify subtype C HIV-I infected adults requiring antiretroviral therapy (ART) and monitoring response to ART in a resource-limited setting. Journal of Translational Medicine 4:33
3) H. Karcher, D. Bohning, R. Downing, S. Mashate, G. Harms. (2006). Comparison of two alternative methods for CD4+ T-cell determination (Coulter manual CD4 count and CyFlow) against standard dual platform flow cytometry in Uganda. Clinical Cytometry, 70B (3):163-69
4) S. Kohreanudom, S. Dettraira. (2012). Evaluation of the Partec Single-Platform Volumetric CyFlow® Counter System for Determining Percentage and Absolute Numbers of CD4 T Lymphocytes in HIV/AIDS Thai Patients. Thai AIDS J, 25:1-10
5) D. Wade, P.A. Diaw, G. Daneau, M. Camara, T.N. Dieye, S. Mboup, L. Kestens. (2013). CD4 T-Cell Enumeration in a Field Setting: Evaluation of CyFlow Counter Using the CD4 Easy Count Kit-Dry and Pima CD4 Systems. PLoS ONE, 8(9): e75484

18 Contacto del fabricante

Fabricante
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Alemanha
www.sysmex-partec.com

Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Número de versión de las instrucciones de uso y fecha de publicación

Revisão: Rev-07_10-09-2020_CN 1563
Publicado por: Sysmex Partec GmbH

Puede consultar la ficha técnica de seguridad de este producto en www.sysmex-partec.com/services.

Instruções de utilização

Identificação do reagente IVD

Nome do produto:

CD4 easy count kit

REF 05-8401

Conteúdo:

Recipiente contendo CD4 mAb PE — REF 05-8401-01
Frasco com tampão sem lise — REF 05-8401-02

1 Especificacao

Especificidade	CD4 humano
Isotipo	IgG1
Conteudo	100 testes
Fluorocromo	PE
λ excitacao (nm)	532 / 488
Maximo de emissao (nm)	578

2 Uso previsto

IVD Para uso no diagnostico in vitro.
O CD4 easy count kit e um teste IVD manual quantitativo com dois componentes, para a marcacao de uma subpopulacao de linfocitos em sangue total EDTA venoso de adulto e enumeracao subsequente com o citometro de fluxo IVD Sysmex Partec CyFlow™ Counter. A concentracao de celulas T CD4 das amostras de sangue e um indicador util para a iniciacao ou o acompanhamento do tratamento de pacientes com VIH positivo em conjugacao com outros resultados laboratoriais e clinicos.
O teste destina-se a ser realizado por profissionais de saude devidamente qualificados.

3 Principio do metodo de analise

Uma alíquota de uma amostra de sangue total com EDTA é misturada com o anticorpo (CD4) conjugado com o fluorocromo numa proporção de 1:1, Apos um tempo de incubacao fixo, o tampao e adicionado e a amostra fica pronta para analise num citometro de fluxo CyFlow™ Counter.
A fonte de luz excita o corante fluorescente ligado com a celula corada e a luz emitida e detetada enquanto um determinado volume de amostra de sangue estiver a fluir atraves do instrumento.
A concentracao da populacao de celulas especificas e calculada pelo software integrado.
Para mais informacoes, consulte as instrucoes de utilizacao do CyFlow™ Counter (CY-S-3022IFUEN).

4 Armazenamento e prazo de validade apos a primeira abertura

1. Armazenamento:
Armazene os reagentes tampao e de anticorpos entre 2 e 8 °C no escuro. Nao congele nem exponha os reagentes a temperaturas elevadas e mantenha-os protegidos da luz solar direta.
2. Prazo de validade apos a primeira abertura:
Feche sempre o frasco apos a utilizacao e utilize pontas de pipeta novas de cada vez que o reagente e amostrado, para evitar a contaminacao.
Guarde os reagentes tampao e de anticorpos entre 2 e 8 °C no escuro. Nao congele nem exponha os reagentes a temperaturas elevadas e mantenha-os protegidos da luz solar direta.
Sob estas condicoes de armazenamento, o CD4 easy count kit ira permanecer estavel ate a data de validade impressa no rotulo do kit.

5 Componentes

O CD4 mAb PE e um anticorpo monoclonal murino fornecido em tampao PBS com 0,2% de BSA e 0,09% de azida de sodio. O tampao sem lise e uma solucao baseada em PBS contendo 0,09% de azida de sodio.

6 Indicio de deterioracao

As solucoes tampao e de anticorpos sao liquidos transparentes. Nao utilize os reagentes caso surja qualquer tipo de turbidez ou contaminacao.
Para questoes relativas ao desempenho ou qualidade do produto recebido, entre em contacto com o representante local da Sysmex para obter suporte tecnico.

7 Precaucoes e advertencias

Os reagentes contem 14 mM de azida de sodio como conservante. A baixa concentracao de azida de sódio nao exige a rotulagem como substancia perigosa, mas e necessario respeitar as precaucoes de seguranca normais relativas ao manuseamento dos produtos quimicos. Consulte a ficha de seguranca para obter uma lista completa das advertencias e precaucoes.

8 Equipamento adicional necessario

Instrumento necessario:	CyFlow™ Counter (REF No.: CY-S-3022)
Equipamento necessario:	Tubos de amostra de 3,5 ml (REF No.: 04-2000) Uma pipeta verificada de 20 µl e pontas de pipeta Uma pipeta verificada de 100-1000 µl e pontas de pipeta Sistema de colheita de sangue venoso com EDTA como anticoagulante Cronometro

9 Preparacao do reagente

CD4 mAb PE (REF No.: 05-8401-01), o reagente de anticorpos esta pronto a usar.
Tampao sem lise (REF No.: 05-8401-02), a solucao tampao esta pronta a usar.

10 Colheita, manuseamento e armazenamento da amostra principal

ADVERTENCIA ⚠ *Todas as amostras biologicas e materiais sao considerados como perigosos e devem ser manuseados como sendo capazes de transmitir infecoes. E necessario aplicar precaucoes de seguranca e procedimentos de manuseamento adequados em conformidade com as leis e regulamentos aplicaveis.*

- A amostra de sangue tem de ser uma amostra de sangue total venoso, recolhida com um sistema de colheita de sangue, que contem EDTA como anticoagulante.
- A amostra de sangue tem de ser transportada num recipiente escuro, protegida da luz e nao exposta a temperaturas elevadas. Um recipiente de transporte adequado e, p. ex., uma caixa de espuma de poliestireno com material de refrigeracao. As melhores condições de transporte seriam inferiores a 25 °C se o transporte não durar mais de 6 horas após a doação. Para transporte e tempo de armazenamento superior a 6 horas, o sangue deve ser mantido a 2 - 8 °C.
- Nao congele e descongele a amostra de sangue.
- A fim de assegurar as melhores condicoes, a amostra de sangue deve ser fresca, ou seja, o tempo de colheita e analise da mesma nao pode ser superior a seis horas.
- As amostras de sangue podem ser utilizadas ate 24 horas apos a colheita, desde que tenham sido armazenadas no frigorifico entre 2 e 8 °C.
- Antes da utilizacao, os tubos de amostra que contem o sangue devem ser invertidos cuidadosamen e 8 a 10 vezes.
- Nao utilize amostras de sangue coagulado.

11 Procedimento de analise

NOTA *A pipetagem reversa é fundamental para a precisão, sobretudo ao dispensar volumes de amostra viscosos e muito reduzidos. Para a pipetagem de sangue total, recomendamos a utilização de uma pipeta eletrónica calibrada pré-programada para operar em modo de pipetagem reversa.*
Se não estiver disponível uma pipeta eletrónica, seguir estas instruções para a pipetagem reversa manual:
- *Premir o botão de operação até ao segundo ponto de retenção. Deixar o botão de operação subir completamente; o excesso da amostra é aspirado para a ponta.*
- *Premir o botão de operação até ao primeiro ponto de retenção para expulsar um volume exato de sangue, deixando o excesso da amostra na ponta.*
- *Deitar a ponta da pipeta com a amostra de sangue em excesso no contentor de lixo hospitalar.*

Ter em atenção que a pipetagem reversa não pode ser usada para anticorpos ou soluções-tampão.

11.1 Coloracao

- Inverta cuidadosamente a amostra de sangue no tubo de colheita de sangue EDTA 8 a 10 vezes.
- Pipete 20 µl de amostra de sangue total EDTA ate ao fundo de um tubo de amostra, evitando deixar um rasto de sangue na parede interior do tubo com a ponta de pipeta. Descarte a ponta da pipeta.
- Adicione 20 µl de CD4 mAb PE diretamente na amostra de sangue e misture cuidadosamente, evitando deixar um rasto de sangue na parede interior do tubo com a ponta de pipeta. Descarte a ponta da pipeta.
- Amostra neste momento nao vortice.
- Incubar a mistura por 15 minutos a 15-30 °C no escuro.
- Adicione 800 µl de tampao sem lise e centrifugue brevemente ou bata cuidadosamente no tubo para misturar a amostra. Descarte a ponta da pipeta.
- Centrifugue brevemente ou bata cuidadosamente no tubo antes de efetuar a analise com o CyFlow™ Counter.

NOTA Apos a adicao do tampao sem lise, as amostras tem de ser analisadas dentro de 2 horas.

11.2 Analise da amostra

Consulte as instrucoes de utilizacao do CyFlow™ Counter para obter informacoes sobre como operar o instrumento antes de analisar as amostras. Os procedimentos de arranque e o procedimento interno de controlo da qualidade tem de ser concluidos com exito antes da analise da amostra.
- Escolha e carregue a configuração para medição de CD4 na barra de menu do CyFlow™ Counter.
- Insira o tubo da amostra com a amostra de sangue preparada na porta de amostragem.
- Inicie a medicao.
- Apos o processamento da amostra, o instrumento para e limpa-se automaticamente.
- Retire e elimine a amostra de sangue de acordo com os procedimentos de seguranca locais do laboratorio em termos de perigo biologico.

Nao inicie uma segunda medicao com o mesmo tubo de amostra. Se for necessaria uma duplicacao ou repeticao da medicao, tera de ser preparado um segundo tubo de amostra tal como descrito em 11.1.

11.3 Aquisicao e analise de dados

A analise de dados com o CyFlow™ Counter so e possivel caso o controlo de qualidade interno do instrumento tenha sido executado com exito.
Consulte as instrucoes de utilizacao do CyFlow™ Counter (CY-S-3022IFUEN) para informacoes sobre o procedimento de arranque e a manutencao devidos do dispositivo, se necessario.
O resultado da contagem de celulas T CD4 sera apresentado no visor.
Se o histograma nao apresentar um pico claro de celulas T coradas positivas CD4, a preparacao e medicao da amostra tem de ser repetidas.
Caso o posicionamento da placa nao seja efetuado com precisao, os resultados serao incorretos.
Caso a concentracao de CD4 seja extremamente baixa numa amostra de sangue do paciente, tenha o cuidado de nao colocar monocitos na placa em vez de celulas coradas positivas CD4.

12 Calculo dos resultados da analise

O calculo da concentracao de celulas faz parte da analise de dados baseada em software, que fornece o resultado em celulas CD4 por amostra de sangue µl, apos cada medicao.
Para mais informacoes, consulte as instrucoes de utilizacao do CyFlow™ Counter (CY-S-3022IFUEN).

13 Interpretacao dos resultados

O vírus da imunodeficiência humana (HIV) é uma das principais razões para a depleção de células CD4, que debilita o sistema imune. Os resultados devem ser interpretados pelos médicos, em conjunção com as diretrizes locais de tratamento do HIV.

14 Procedimento de controlo

Deve ser realizado um controlo da qualidade apropriado de acordo com regulamentos laboratoriais nacionais.
Encontra-se disponivel no mercado uma variedade de amostras de sangue estabilizado, mas poucas sao adequadas para a utilizacao de certos citometros de fluxo.
Se nao for cumprido o criterio de aceitacao do material de controlo individual, nao avance para o teste da amostra do paciente e entre em contacto com o seu representante local da Sysmex.
Para mais informacoes acerca do uso de material de controlo adequado, entre em contacto com o seu representante local da Sysmex.

15 Caracteristicas de desempenho

15.1 Especificidade

O anticorpo monoclonal do rato MEM-241 reconhece o antigenio CD4 humano, uma glicoproteina transmembrana (55 kDa) da familia do supergene da imunoglobulina, presente num subconjunto de linfocitos T (celulas T .auxiliares/indutorasg) e expressa a um nivel inferior em monocitos, granulocitos e macrofagos do tecido. Conferencia sobre HCDM (o anterior HLDA VIII), maio de 2006, Quebeque, Canada; codigo WS M241

15.2 Capacidade de detecao

A avaliacao para Limite de valores em branco (LoB), Limite de detecao (LoD) e Limite de quantificacao (LoQ) foi realizada de acordo com as especificacoes da diretriz CLSI EP17-A2.
LoB: ≤50% de LoD
LoD: ≤20 celulas CD4/µl
LoQ: ≤40 celulas CD4/µl

15.3 Repetibilidade

Variacao intra e entre ensaios:
Os valores CV sao inferiores a 10% para valores CD4 de 200 celulas T CD4/µl ou superiores.
Os valores CV sao inferiores a 15% para valores absolutos de CD4 inferiores a 200 celulas T CD4/µl.

15.4 Linearidade

A gama de medicao do ensaio e linearidade da medicao sao validas ao longo da gama:
CD4 absoluto: 40-2500 celulas T CD4/µl

15.5 Veracidade

Uma amostra de sangue total EDTA foi corada com o CD4 easy count kit e analisada no CyFlow™ Counter. Os valores de CD4 absoluto foram comparados com os resultados de um BD FACSCalibur usando BD Tritest CD3/CD4/CD45 e tubos BD Trucount, tendo-se revelado um desvio de -22,56% entre ambos os metodos.

16 Limitacoes

CUIDADO ⚠ *Os kits Sysmex Partec CD4 easy count (05-8401) nao foram validados para utilizacao com pacientes pediatricos e adolescentes.*

CUIDADO ⚠ *Os medicoes de paciente de CD4 e de CD4% feitas com os ensaios Sysmex Partec nao devem ser usadas de forma independente com outros metodos do fabricante para determinar CD4 e CD4%. Use os ensaios apenas com os instrumentos Sysmex Partec. Os valores obtidos usando outros equipamentos ou ensaios podem nao ser intercambiaveis.*

A Foram testadas diversas substancias endogenas e exogenas relativamente a diretriz CLSI EP07-A2 para identificar possiveis efeitos de interferencia que possam exercer influencia sobre a analise da amostra de sangue.
Finalmente, observouse . 18% de hemolise (induzida usando o protocolo recomendado na CLSI EP07-A2, Apendice G, G1 - Procedimento de Choque Osmotico) para demonstrar um efeito de interferencia. Por esse motivo, deverao ser rejeitadas amostras hemolisadas. Para outras questoes relativas ao desempenho do produto, entre em contacto com o seu representante local da Sysmex.

17 Referencias bibliograficas

1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondiere, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
2) L. Zijenah, G. Kadzirange, S. Madzime, M. Borok, C. Mudiwa, O. Tobaiwa, M. Mucheche, S. Rusakaniko, D. Katzenstein. (2006). Affordable flow cytometry for enumeration of absolute CD4+ T-lymphocytes to identify subtype C HIV-I infected adults requiring antiretroviral therapy (ART) and monitoring response to ART in a resource-limited setting. Journal of Translational Medicine 4:33
3) H. Karcher, D. Bohning, R. Downing, S. Mashate, G. Harms. (2006). Comparison of two alternative methods for CD4+ T-cell determination (Coulter manual CD4 count and CyFlow) against standard dual platform flow cytometry in Uganda. Clinical Cytometry, 70B (3):163-69
4) S. Kohreanudom, S. Dettraira. (2012). Evaluation of the Partec Single-Platform Volumetric CyFlow® Counter System for DeterminingPercentage and Absolute Numbers of CD4 T Lymphocytes in HIV/AIDS Thai Patients. Thai AIDS J, 25:1-10
5) D. Wade, P.A. Diaw, G. Daneau, M. Camara, T.N. Dieye, S. Mboup, L. Kestens. (2013). CD4 T-Cell Enumeration in a Field Setting: Evaluation of CyFlow Counter Using the CD4 Easy Count Kit-Dry and Pima CD4 Systems. PLoS ONE, 8(9): e75484

18 Contacto do fabricante

Fabricante
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Alemanha
www.sysmex-partec.com

Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Numero de versao das instrucoes de utilizacao e data da pu blicacao

Revisao : Rev-07_10-09-2020_CN 1563
Publicado por: Sysmex Partec GmbH

A ficha de seguranca deste produto esta disponivel em www.sysmex-partec.com/services.

Инструкция по эксплуатации

Идентификация реагента для диагностики in vitro (IVD)

Наименование продукта

CD4 easy count kit

REF 05-8401

Содержимое

Ампула, содержащая CD4 mAb PE — REF 05-8401-01
Флакон, содержащий нелизирующий буфер — REF 05-8401-02

1 Спецификация

Специфичность	CD4 человека
Изотип	IgG1
Содержимое	100 тестов
Флуорохром	PE
λ возбуждение (нм)	532 / 488
Максимальная эмиссия (нм)	578

2 Предполагаемое использование

IVD Для использования в диагностике in vitro.
Набор CD4 easy count kit – это двухкомпонентный набор, предназначенный для ручного количественного анализа in vitro, используемый для мечения субпопуляций лимфоцитов в цельной венозной крови с ЭДТА взрослых пациентов и их последующего подсчета с помощью проточного цитометра для диагностики in vitro CyFlow™ Counter компании Sysmex Partec.
Концентрация CD4 Т-клеток в образцах крови является полезным показателем для начала или продолжения лечения ВИЧ-положительных пациентов наряду с другими лабораторными и клиническими данными.
Анализ должен проводиться обученными специалистами здравоохранения.

3 Принцип метода подсчета

Аликвоту образца цельной крови EDTA смешивают с антителом (CD4), конъюгированным с флуорохромом, в соотношении 1:1, После инкубирования в течение фиксированного времени добавляют буферный раствор, после чего образец готов для анализа на проточном цитометре CyFlow™ Counter.
Источник света активирует флуоресцентный краситель, которым мечена клетка, и испускаемый красителем свет определяется во время анализа образца крови определенного объема в приборе.
Концентрация клеток определенной популяции рассчитывается с помощью интегрированного программного обеспечения. Для получения дополнительной информации см. ИЭ цитометра CyFlow™ Counter (CY-S-3022IFUEN).

4 Хранение и срок годности после первого вскрытия

1. Хранение.
Антитела и буферные реагенты следует хранить при температуре 2 – 8°C в темном месте. Реагенты нельзя замораживать, подвергать воздействию высоких температур и прямых солнечных лучей.
2. Срок годности после первого вскрытия.
Следует всегда закрывать флакон после использования и каждый раз при взятии реагента пользоваться новым наконечником для пипетки с целью предотвращения контаминации.
Антитела и буферные реагенты следует хранить при температуре 2 – 8°C в темном месте. Реагенты нельзя замораживать, подвергать воздействию высоких температур и прямых солнечных лучей.
При таких условиях хранения реагенты набора CD4 easy count kit будут стабильны до окончания срока годности, указанного на его этикетке.

5 Компоненты

CD4 mAb PE — это мышиные моноклональные антитела в фосфатно-солевом буфере (PBS), содержащем 0,2 % БСА и 0,09 % азида натрия. Нелизирующий буфер представляет собой раствор на основе PBS, содержащий 0,09 % азида натрия.

6 Признаки ухудшения качества

Антитела и буферные растворы представляют собой прозрачную жидкость. Не следует использовать реагенты при появлении любого рода помутнения или признаков контаминации. При наличии вопросов о характеристиках или качестве продукта обращайтесь в службу технической поддержки местного представительства компании Sysmex.

7 Предупреждения и меры предосторожности

Реагенты содержат 14 мМ азида натрия в качестве консерванта. Низкая концентрация азида натрия не требует маркировки опасностей, однако при их использовании следует соблюдать стандартные меры предосторожности по безопасному обращению с химическими веществами. Полный перечень предупреждений и мер предосторожности приведен в паспорте безопасности (ПБ) продукта.

8 Дополнительное требуемое оборудование

Требуемый прибор:	CyFlow™ Counter (REF No.: CY-S-3022)
Требуемое оборудование:	пробирки объемом 3,5 мл (REF No.: 04-2000) поверенная пипетка объемом 20 мкл и наконечники для пипетки поверенная пипетка объемом 100–1000 мкл и наконечники для пипетки система для сбора венозной крови с ЭДТА в качестве антикоагулянта секундомер

9 Приготовление реагента

CD4 mAb PE (REF No.: 05-8401-01), Реагент с антителами готов к использованию.
нелизирующий буфер (REF No.: 05-8401-02), Буферный раствор готов к использованию.

10 Взятие основного образца, обращение с ним и его хранение

ПРЕДУПРЕЖДЕНИЕ ⚠ *Все биологические образцы и материалы считаются биологически опасными, с ними следует обращаться как с потенциальным источником инфекции. При обращении с ними следует соблюдать надлежащие меры предосто-рожности и требования безопасности в соответствии с применимыми законами и нормативами.*

- Образцом должен служить образец цельной венозной крови, взятый с помощью системы для сбора крови, содержащей ЭДТА в качестве антикоагулянта.
- Образец крови следует транспортировать в непрозрачном контейнере, защищать от воздействия света и не подвергать воздействию высоких температур. Подходящим транспортным контейнером является, например, пенопластовый (Styrofoam) контейнер с охлаждающим материалом. Лучшие транспортные условия будут ниже 25 °C, если транспорт не займет больше 6 часов после пожертвования. Для транспортировки и хранения более 6 часов кровь должна храниться при температуре 2-8 °C.
- Нельзя замораживать и размораживать образец крови.
- Для получения наилучших результатов образец крови должен быть свежим, т. е. между его взятием и анализом должно пройти не более шести часов.
- Образцы крови можно использовать в течение 24 часов после взятия, если они хранятся в холодильнике при температуре 2 – 8°C.
- Перед началом анализа пробирку с образцом крови следует аккуратно перевернуть от 8 до 10 раз.
- Запрещается использование свернувшихся образцов крови.

11 Процедура анализа

ПРИМЕЧАНИЕ *Обратное пипетирование крайне важно для обеспечения точности, особенно при дозировании проб очень малого объёма. Рекомендуется использовать калиброванную электронную пипетку, запрограммированную на работу в режиме обратного пипетирования.*
Если электронная пипетка недоступна, выполните обратное пипетирование вручную следующим образом:
- *нажмите на кнопку управления до второго упора. Дайте кнопке управления полностью подняться, при этом избыток пробы втягивается в кончик.*
- *нажмите на кнопку управления до первого упора, чтобы выдавить точный объём крови, оставив избыток пробы в кончике.*
- *Выбросьте кончик пипетки с избытком пробы крови в контейнер для медицинских отходов.*

Обратите внимание, что обратное пипетирование нельзя использовать для растворов антител и буферных растворов.

11.1 Окрашивание

- Аккуратно переверните пробирку с ЭДТА и образцом крови от 8 до 10 раз.
- Перенесите пипеткой 20 мкл образца цельной крови с ЭДТА на дно пробирки для анализа, не оставляя наконечником пипетки следов крови на внутренней стенке пробирки. Отбросьте наконечник пипетки.
- Добавьте 20 мкл раствора антитела CD4 mAb PE непосредственно в образец крови и аккуратно перемешайте, не оставляя наконечником пипетки следов крови на внутренней стенке пробирки. Отбросьте наконечник пипетки.
- Образец в это время не завихрения.
- Инкубируйте смесь в течение 15 минут при температуре 15-30 °C в темноте.
- Добавьте 800 мкл нелизирующего буфера и перемешайте (недолго) содержимое пробирки вихревым способом или аккуратно постучите по пробирке. Отбросьте наконечник пипетки.
- Перед анализом в цитометре CyFlow™ Counter перемешайте содержимое пробирки вихревым способом или аккуратно постучите по пробирке.

ПРИМЕЧАНИЕ *После добавления нелизирующего буфера образцы должны быть проанализированы в течение 2 часов.*

11.2 Анализ образца

Для получения информации о работе с цитометром CyFlow™ Counter (CY-S-3022IFUEN) перед анализом образцов см. его ИЭ. Перед анализом образца следует успешно завершить предпусковые процедуры и процедуру внутреннего контроля качества.
- Выберите и загрузите конфигурацию для измерения CD4 из строки меню CyFlow™ Counter.
- Вставьте пробирку с приготовленным образцом крови в соответствующий держатель.
- Начните анализ.
- После анализа образца прибор прекращает работу и очищается автоматически.
- Извлеките и утилизируйте образец крови в соответствии с местными правилами по биологической безопасности при работе в лабораториях.

Не проводите повторный анализ с использованием той же самой пробирки с образцом. Если нужно выполнить дублирующее или повторное измерение, вторую пробирку для анализа подготовьте согласно п. 11.1.

11.3 Сбор и анализ данных

Анализ данных с помощью цитометра CyFlow™ Counter возможен только при успешном выполнении процедуры внутреннего контроля качества прибора.
Информация, необходимая для правильного запуска и технического обслуживания прибора, содержится в ИЭ цитометра CyFlow™ Counter (CY-S-3022IFUEN).
Результат определения количества CD4-Т-клеток будет представлен на экране. Если на гистограмме отсутствует четкий пик положительно окрашенных CD4-Т-клеток, следует приготовить новый испытуемый образец и повторить анализ.
Результаты будут неверными, если позиционирование области клеток будет выполнено не точно.
Следует с осторожностью интерпретировать результаты анализа и не относить моноциты к положительно окрашенным CD4-клеткам, если концентрация CD4-клеток в образце крови пациента является очень низкой.

12 Расчет результатов анализа

Расчет концентрации клеток выполняется в рамках анализа данных программным обеспечением, что дает результат определения количества CD4-клеток на 1 мкл образца крови после каждого анализа.
Для получения дополнительной информации см. ИЭ цитометра CyFlow™ Counter (CY-S-3022IFUEN).

13 Интерпретация результатов

Вирус иммунодефицита человека (ВИЧ) является одной из основных причин истощения клеток CD4, которая дебиллирует систему im-mune. Рецензии следует интерпретировать врачами в соответствии с местными рекомендациями по лечению ВИЧ.

14 Контрольная процедура

Следует проводить надлежащий контроль качества согласно национальным нормативным требованиям к проведению лабораторных исследований.
В продаже присутствуют различные стабилизированные образцы крови, однако только некоторые из них подходят для конкретных типов проточных цитометров.
В случае несоблюдения критерия приемлемости для отдельного контрольного материала не выполняйте исследование образца пациента и обратитесь к местному представителю компании Sysmex.
За дополнительной информацией об использовании подходящего контрольного материала обратитесь к местному представителю компании Sysmex.

15 Рабочие характеристики

15.1 Специфичность

Мышиное моноклональное антитело MEM-241 распознает антиген CD4 человека, транс мембранный гликопротеин (55 кДа), входящий в семейство супергена иммуноглобулинов, который экспрессируется на поверхности клеток одной из субпопуляций Т-лимфоцитов (Т-хелперы/Т-индукторы), а также, в меньших количествах, на поверхности моноцитов, тканевых макрофагов и гранулоцитов. Рабочее совещание по дифференцировочным молекулам клеток человека — HCDM (ранее VIII семинар по дифференцировочным антигенам лейкоцитов человека — HLDA), май 2006 г., Квебек, Канада; код WS M241

15.2 Способность обнаружения

Выполнялась оценка для предела холостой пробы (LoB), предела обнаружения (LoD) и предела количественного определения (LoQ) согласно спецификациям, указанным в руководстве CLSI EP17-A2.
LoB: ≤50% от LoD
LoD: ≤20 CD4-клеток/мкл
LoQ: ≤40 CD4-клеток/мкл

15.3 Воспроизводимость

Вариабельность в пределах одного и нескольких анализов:
Значения коэффициента вариации (CV) составляют менее 10 % для количества CD4-клеток, равного или превышающего 200 CD4-Т-клеток на 1 мкл.
Значения коэффициента вариации (CV) составляют менее 15 % для абсолютного количества CD4-клеток ниже 200 CD4-Т-клеток на 1 мкл.

15.4 Линейность

Диапазон измерений анализа и линейность анализа валидны в пределах следующего диапазона: абсолютное количество CD4-клеток: 40–2500 CD4-Т-клеток на 1 мкл;

15.5 Правильность

Образцы цельной крови с ЭДТА окрашивали с помощью набора CD4 easy count kit и анализировали на цитометре CyFlow™ Counter. Абсолютное количество CD4-клеток сравнивали с результатами анализа на цитометре BD FACSCalibur с использованием реагентов BD Tritest CD3/CD4/CD45 и пробирок BD Trucount. Систематическое расхождение между методами составило -22,56 %.

16 Ограничения

ВНИМАНИЕ ⚠ *Наборы Sysmex Partec CD4 easy count (05-8401) не были валидированы для использования у пациентов детского и подросткового возраста.*

ВНИМАНИЕ ⚠ *Измерения CD4 и CD4%, проведённые при помощи анализов Sysmex Partec, не следует использовать независимо с методами определения CD4 и CD4% от других производителей. Используйте анализы только с приборами Sysmex Partec. Значения, полученные с использованием других приборов или анализов, могут быть неравнозначными.*

Различные эндогенные и экзогенные вещества были проверены в соответствии с руководством CLSI EP07-A2 на возможность искажения результатов анализа образца крови. В ≥ 18 % случаев гемолиза (вызванного применением протокола, рекомендованного в CLSI EP07-A2, приложение G, G1: метод осмотического шока) наблюдалось искажение результатов. По этой причине не следует использовать гемолизированные образцы. При наличии дополнительных вопросов о характеристиках продукта обратитесь к местному представителю компании Sysmex.

17 Литература

1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondière, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
2) L. Zijenah, G. Kadzirange, S. Madzime, M. Borok, C. Mudiwa, O. Tobaiwa, M. Mucheche, S. Rusakaniko, D. Katzenstein. (2006). Affordable flow cytometry for enumeration of absolute CD4+ T-lymphocytes to identify subtype C HIV-I infected adults requiring antiretroviral therapy (ART) and monitoring response to ART in a resource-limited setting. Journal of Translational Medicine 4:33
3) H. Karcher, D. Bohning, R. Downing, S. Mashate, G. Harms. (2006). Comparison of two alternative methods for CD4+ T-cell determination (Coulter manual CD4 count and CyFlow) against standard dual platform flow cytometry in Uganda. Clinical Cytometry, 70B (3):163-69
4) S. Kohreanudom, S. Dettraira. (2012). Evaluation of the Partec Single-Platform Volumetric CyFlow® Counter System for Determining Percentage and Absolute Numbers of CD4 T Lymphocytes in HIV/AIDS Thai Patients. Thai AIDS J, 25:1-10
5) D. Wade, P.A. Diaw, G. Daneau, M. Camara, T.N. Dieye, S. Mboup, L. Kestens. (2013). CD4 T-Cell Enumeration in a Field Setting: Evaluation of CyFlow Counter Using the CD4 Easy Count Kit-Dry and Pima CD4 Systems. PLoS ONE, 8(9): e75484

18 Контактная информация производителя

Производитель
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Германия
www.sysmex-partec.com

Тел+49 3581 8746 0
Факс+49 3581 8746 70
Эл. почта:info@sysmex-partec.com

19 Номер редакции и дата выпуска ИЭ

Редакция: ред -07_10-09-2020_CN 1563
Выпущено: Sysmex Partec GmbH

Паспорт безопасности данного продукта доступен на веб-сайте www.sysmex-partec.com/services.

2.2 CD4% easy count kit IFU (product code 05-8405)

Identification of the IVD reagent
Product name:
CD4% easy count kit
REF 05-8405

Content:

Vial containing CD4 mAb PE	REF 05-8405-01
Vial containing CD45 mAb PE-Cy5	REF 05-8405-02
Bottle containing Buffer 1	REF 05-8405-03
Bottle containing Buffer 2	REF 05-8405-04

1 Specification

Specificity	Human CD4	Human CD45
Isotype	IgG1	IgG1
Content	100 tests	100 tests
Fluorochrome	PE	PE-Cy5
λ excitation (nm)	532 / 488	532 / 488
Emission maximum (nm)	578	670

2 Intended Use
IVD For In Vitro Diagnostic Use.

The CD4% easy count kit is a manual, four-component, quantitative IVD test for labelling of leukocytes and a subpopulation of lymphocytes in adult venous EDTA whole blood, which can then be enumerated with the Sysmex Partec CyFlow™ Counter IVD flow cytometer. The CD4 T cell concentration and CD4% of lymphocytes in blood samples are useful indicators for the initiation or follow-up of treatment for HIV positive patients in conjunction with other laboratory and clinical findings.
The test is intended to be performed by trained healthcare professionals.

3 Principle of the examination method
An aliquot of an EDTA whole-blood sample is mixed with two antibodies (CD4 and CD45), each conjugated to a different fluorochrome for labelling dedicated cell populations. After a fixed incubation time, the two buffer solutions are added and the sample is ready for analysis on the CyFlow™ Counter flow cytometer. The light source excites the fluorescent dye linked with the stained cell and the emitted light is detected while a precise volume of blood sample is running through the instrument. The concentration of the dedicated cell populations is calculated by the integrated software.
For further information, please refer to the IFU of CyFlow™ Counter (CY-S-3022IFUEN).

4 Storage and shelf life after first opening
1. Storage:
Store the antibody and buffer reagents at 2 - 8°C in the dark. Do not freeze or expose the reagents to elevated temperatures and keep them away from direct sunlight.
2. Shelf life after first opening:
Always close the bottle after use and use new pipette tips each time the reagent is sampled to avoid contamination.
Under these storage conditions, the CD4% easy count kit will be stable until the expiration date printed on the kit label.

5 Components
CD4 mAb PE and CD45 mAb PE-Cy5 are murine monoclonal antibodies supplied in PBS buffer with 0.2% BSA and 0.09% sodium azide. Buffer 1 and Buffer 2 are PBS-based solutions containing 0.09% sodium azide.

6 Evidence of deterioration
The antibody and buffer solutions are clear liquids. Do not use the reagents if there is the appearance of any kind of turbidity or contamination.
For questions regarding the performance or quality of the product, please contact your local Sysmex representative.

7 Precaution and warnings
Reagents contain 14 mM sodium azide as a preservative. The low concentration of sodium azide does not require hazard labelling, but the normal safety precautions for the handling of chemicals must be observed. Refer to the Safety Data Sheet (SDS) for a complete list of warnings and precautions.

8 Additional required equipment

Instrument requirement:	CyFlow™ Counter (REF No.: CY-S-3022)
Required apparatus:	Sample Tubes 3.5 ml (REF No.: 04-2000) A verified pipette 10 µl and pipette tips A verified pipette 20 µl and pipette tips A verified pipette 100 – 1000 µl and pipette tips Venous blood collection system with EDTA as anticoagulant Stop watch

9 Reagent preparation
CD4 mAb PE (REF No.: 05-8405-01), the antibody reagent is ready to use.
CD45 mAb PE-Cy5 (REF No.: 05-8405-02), the antibody reagent is ready to use.
Buffer 1 (REF No.: 05-8405-03), the buffer solution is ready to use.
Buffer 2 (REF No.: 05-8405-04), the buffer solution is ready to use.

10 Primary sample collection, handling and storage
WARNING ⚠ *All biological specimens and material are considered as biohazards and should be handled as if capable of transmitting infection. Appropriate safety precautions and handling procedures must be applied in accordance with applicable laws and regulations.*

- The blood sample must be a venous, whole-blood sample, collected with a blood collection system which contains EDTA as anticoagulant.
- The blood sample must be transported in a dark container, protected from light and not exposed to elevated temperatures. A suitable transport container is, for example, a Styrofoam box with cooling material. Best transport conditions would be below 25 °C if transport will not take more than 6 hours after donation.
- For transport and storage time more than 6 hours, blood must be kept at 2 - 8 °C.
- Do not freeze and thaw the blood sample.
- For best conditions, the blood sample should be fresh, i.e. not more than six hours should have elapsed between sample collection and analysis.
- Blood samples can be used up to 24 hours after drawing if they are stored in the fridge at 2 - 8 °C.
- Before use, the sample tubes containing the blood must be inverted gently 8 to 10 times.
- Do not use clotted blood samples.

11 Examination procedure
NOTICE *Reverse pipetting is critical to accuracy especially when dispensing viscous and very small sample volumes. For whole blood pipetting, we recommend using a calibrated electronic pipette which is preprogrammed to operate in the reverse pipetting mode.*
If an electronic pipette is not available, follow these instructions for manual reverse pipetting:
- *Depress the operating button to the second stop. Let the operating button move up completely, excess sample is drawn up into the tip.*
- *Depress the operating button to the first stop to expel a precise volume of blood, leaving excess sample in the tip.*
- *Discard pipette tip with excess blood sample in the tip into medical waste container.*

Please note, you must not use reverse pipetting for antibody or buffer solutions.

11.1 Staining
- Invert the blood sample in the EDTA blood collection tube gently 8 to 10 times.
- Pipette 20 µl EDTA whole blood sample to the bottom of a sample tube, avoid leaving a trail of blood on the inner tube wall from pipette tip. Discard the pipette tip.
- Add 10 µl CD4 mAb PE directly into the blood sample and mix it gently, avoid leaving a trail of blood on the inner tube wall from pipette tip. Discard the pipette tip.
- Add 10 µl CD45 mAb PE-Cy5 directly into the blood sample and mix it gently, avoid leaving a trail of blood on the inner tube wall from pipette tip. Discard the pipette tip.
- Incubate the mixture for 15 minutes at 15 - 30 °C in the dark.
- Add 400 µl Buffer 1 and vortex briefly, or tap tube gently in order to mix the sample. Discard the pipette tip.
- Add 400 µl Buffer 2 and vortex briefly, or tap tube gently in order to mix the sample. Discard the pipette tip.
- Vortex briefly, or tab tube gently before analysing with the CyFlow™ Counter.

NOTICE *After addition of Buffer 1, samples can be stored for up to 2 hours at 2 - 8 °C in the dark. Do not use the sample later than that or if not prepared accordingly.*
After addition of Buffer 2, samples must be analysed within 10 minutes.

11.2 Sample analysis
Please refer to the IFU of the CyFlow™ Counter (CY-S-3022IFUEN) for how to operate the instrument before analysing the sample. The start-up procedures and the internal quality control procedure must be successfully completed before sample analysis.
- Choose and load the configuration for CD4% measurement from the menu bar of CyFlow™ Counter.
- Insert the sample tube with the prepared blood sample into the sample port.
- Start measurement.
- After sample run, the instrument stops and cleans automatically.
- Remove and dispose of blood sample in accordance with local laboratory biohazard safety procedures.

Do not start a second measurement with the same sample tube. If a duplicate or repeat measurement is required, a second sample tube has to be prepared as per 11.1.

11.3 Data acquisition and analysis
Data analysis with the CyFlow™ Counter is only possible if the internal quality control of the instrument was successful.
Please refer to the IFU of the CyFlow™ Counter (CY-S-3022IFUEN) for start-up procedure and maintenance of the device, if necessary.
The CD4 T-cell counting result, CD4 percentage and total count of lymphocytes will be displayed on the screen. For results to be reliable there must be good separation of cell clusters as shown in Figure 1. If the clusters of stained cells towards the debris signals in the CD45 – SSC dot plot are not clearly separable (see Figure 2), or the clusters of CD4 positive cells, CD4 negative cells and monocytes in the CD4 – SSC dot plot are not clearly separable, the sample preparation and measurement must be repeated.

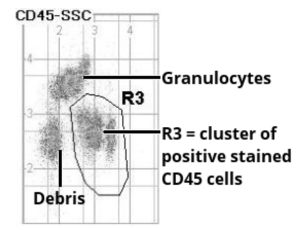


Figure 1: Example of well separated clusters of granulocytes, other CD45 positive stained leucocytes and debris signals.

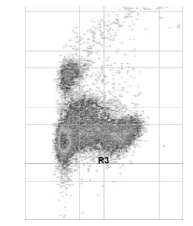


Figure 2: Example of bad separation between debris and CD45 positive stained leucocytes. This sample is not analysable.

Results will be incorrect if gate positioning of CD45 positive stained cells (R3) is not done precisely.

12 Calculation of examination results
Calculation of the cell concentration is part of the software-based data analysis, which provides the result in CD4%, and CD4+ cells per µl blood sample after each measurement. For further information, please refer to the IFU of the CyFlow™ Counter (CY-S-3022IFUEN).

13 Interpretation of results
Human Immunodeficiency Virus (HIV) is one of the main reasons for CD4 cell depletion, which debilates the immune system. Results should be interpreted by physicians, in conjunction with locally applicable HIV treatment guidelines.

14 Control procedure
Appropriate quality control should be performed according to local and national regulations. A variety of stabilized blood samples are available on the market, only a few are suitable when using certain flow cytometers. If the acceptance criterion of the individual control material is not met, do not proceed with patient specimen testing and contact your local Sysmex representative. For further information about the use of adequate control material, please contact your local Sysmex representative.

15 Performance characteristics
15.1 Specificity
The antibody MEM-241 recognizes CD4 antigen, a 55 kDa transmembrane glycoprotein expressed on a subset of T lymphocytes ("helper" T-cells) and on monocytes, tissue macrophages and granulocytes. HCDM (former HLDA VIII) Meeting, May 2006, Québec, Canada; WS Code M241
The antibody MEM-28 reacts with all alternative forms of human CD45 antigen (Leukocyte Common Antigen), a 180-220 kDa single chain type I transmembrane protein expressed at high level on all cells of hematopoietic origin, except erythrocytes and platelets. HLDA III; WS Code NL 833a

15.2 Detection ability
The evaluation for Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) was carried out according to the specifications in the guideline CLSI EP17-A2.
LoB: ≤50% of LoD
LoD: ≤20 CD4 cells/µl
LoQ: ≤40 CD4 cells/µl

15.3 Repeatability
Intra- and inter-assay variation:
CV values are less than 10% for CD4 values of 200 CD4 T-cells/µl or above.
CV values are less than 10% for CD4% values for 25% or above.
CV values are less than 15% for CD4 absolute values below 200 CD4 T-cells/µl.
SD values are less or equal to 2.5% for CD4% values below 25%.

15.4 Linearity
The measuring range of the assay and linearity of the measurement are valid over the range:
CD4 absolute: 40-2500 CD4 T-cells/µl
CD4%: 4 – 60%

15.5 Trueness
EDTA whole blood specimen were stained with CD4% easy count kit and analysed on the CyFlow™ Counter (CyView™ 2.11). CD4 absolute and CD4% values were compared with results from a BD FACSCalibur using BD Tritest CD3/CD4/CD45 and BD Trucount tubes and showed a bias of -22.56% (CD4 absolute) and +2.1% (CD4%) between both methods.

16 Limitations
CAUTION ⚠ *Sysmex Partec CD4% easy count kits (05-8405) have not been validated for use with pediatric and adolescent patients.*

CAUTION ⚠ *Patient measurements of CD4 and CD4% made using Sysmex Partec assays should not be used alongside measurements obtained from other manufacturer's methods of determining CD4 and CD4%. Use the assays only with Sysmex Partec instruments. Values obtained using other equipment or assays are not interchangeable.*

Different endogenous and exogenous substances were tested regarding the CLSI EP07-A2 guideline for possible interfering effects which could have an influence to the blood sample analysis.
Finally, ≥ 18% haemolysis (induced by using the protocol recommended in the CLSI EP07-A2, Appendix G, G1 - Osmotic Shock Procedure) was observed to show an interfering effect. For that reason, haemolyzed samples should be rejected. For CD4 and CD4% determination in whole blood samples with CyFlow™ Counter assays, no other anticoagulant than EDTA should be used.
For further questions regarding the performance of the product, please contact your local Sysmex representative.

17 Literature references
1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondière, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
2) L. Zijenah, G. Kadzirange, S. Madzime, M. Borok, C. Mudiwa, O. Tobaiwa, M. Mucheche, S. Rusakaniko, D. Katzenstein. (2006). Affordable flow cytometry for enumeration of absolute CD4+ T-lymphocytes to identify subtype C HIV-I infected adults requiring antiretroviral therapy (ART) and monitoring response to ART in a resource-limited setting. Journal of Translational Medicine 4:33
3) H. Karcher, D. Bohning, R. Downing, S. Mashate, G. Harms. (2006). Comparison of two alternative methods for CD4+ T-cell determination (Coulter manual CD4 count and CyFlow) against standard dual platform flow cytometry in Uganda. Clinical Cytometry, 70B (3):163-69
4) S. Kohreanudom, S. Dettraira. (2012). Evaluation of the Partec Single-Platform Volumetric CyFlow® Counter System for Determining Percentage and Absolute Numbers of CD4 T Lymphocytes in HIV/AIDS Thai Patients. Thai AIDS J, 25:1-10
5) D. Wade, P.A. Diaw, G. Daneau, M. Camara, T.N. Dieye, S. Mboup, L. Kestens. (2013). CD4 T-Cell Enumeration in a Field Setting: Evaluation of CyFlow Counter Using the CD4 Easy Count Kit-Dry and Pima CD4 Systems. PLoS ONE, 8(9): e75484

18 Contact of manufacturer
Manufacturer
Sysmex Partec GmbH
Arndtstraße 11 a-b
02828 Görlitz, Germany
www.sysmex-partec.com
Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Version number of IFU and date of issue
Revision: Rev-07_10-09-2020_CN 1563
Issued by: Sysmex Partec GmbH

Safety Data Sheet for this product is available at www.sysmex-partec.com/services.

Identifikation des IVD-Reagens
Produktname:
CD4% easy count kit
REF 05-8405

Inhalt:

Ampulle mit CD4 mAb PE	REF 05-8405-01
Ampulle mit CD45 mAb PE-Cy5	REF 05-8405-02
Flasche mit Puffer 1	REF 05-8405-03
Flasche mit Puffer 2	REF 05-8405-04

1 Specification

Spezifität	Humanes CD4	Humanes CD45
Isotyp	IgG1	IgG1
Inhalt	100 Tests	100 Tests
Fluorochrom	PE	PE-Cy5
λ Anregung (nm)	532 / 488	532 / 488
Emissionsmaximum (nm)	578	670

2 Zweckbestimmung
IVD In-vitro-Diagnostikum.

Das **CD4% easy count kit** ist ein manueller, quantitativer 4-Komponenten-In-vitro-Test für die Markierung von Leukozyten und einer Lymphozyten-Untergruppe in EDTA-Vollblutproben von Erwachsenen, die anschließend mit dem Sysmex Partec IVD-Flowzytometer CyFlow™ Counter ausgezählt werden können. Die Konzentration der CD4-positiven T-Helferzellen sowie der Anteil der CD4-Helferzellen an den Lymphozyten (CD4%) in Blutproben sind in Verbindung mit anderen Laborwerten und klinischen Befunden nützliche Indikatoren für die Einleitung der Behandlung HIV-positiver Patienten und die Nachsorge.
Der Test ist nur von geschulten medizinischen Fachkräften durchzuführen.

3 Prinzip des Untersuchungsverfahrens
Ein Aliquot einer EDTA-Vollblutprobe wird mit zwei Antikörpern gemischt (CD4 und CD45), wobei jeder mit einem anderen Fluorochrom zur Markierung von spezifischen Zellpopulationen konjugiert ist. Nach einer bestimmten Inkubationszeit werden die beiden Pufferlösungen hinzugegeben; nun kann die Analyse der Probe im CyFlow™ Counter-Flowzytometer erfolgen. Die Lichtquelle regt den Fluoreszenzfarbstoff an, der mit der gefärbten Zelle verbunden ist, und das emittierte Licht wird detektiert, während ein genaues Volumen der Blutprobe durch das Instrument läuft. Die Konzentration der spezifischen Zellpopulationen wird von der integrierten Software berechnet.
Weitere Informationen entnehmen Sie bitte dem Benutzerhandbuch des CyFlow™ Counter (CY-S-3022IFU-EN).

4 Lagerung und Haltbarkeitsdauer nach dem ersten Öffnen
1. Lagerung:
Das Antikörper- und Pufferreagens bei 2 - 8 °C im Dunkeln aufbewahren. Nicht einfrieren oder erhöhten Temperaturen aussetzen. Vor direkter Sonneneinstrahlung schützen
2. Haltbarkeitsdauer nach dem ersten Öffnen:
Die Flasche nach jedem Gebrauch wieder verschließen und für jede weitere Entnahme des Reagens neue Pipettenspitzen verwenden, um eine Verunreinigung zu vermeiden.
Unter Einhaltung der oben genannten Lagerbedingungen ist das CD4% easy count kit bis zum Verfallsdatum auf dem Etikett des Kits stabil.

5 Bestandteile
CD4 mAb PE und CD45 mAb PE-Cy5 sind murine monoklonale Antikörper in PBS-Puffer mit 0,2 % BSA und 0,09 % Natriumazid. Puffer 1 und Puffer 2 sind Lösungen auf PBS-Basis, die 0,09 % Natriumazid enthalten.

6 Anzeichen von Verderb
Die Antikörper- und Pufferlösungen sind klare Flüssigkeiten. Die Reagenzien nicht verwenden, falls Trübungen oder Verunreinigungen auftreten. Bei Fragen zur Leistung oder Qualität des Produkts wenden Sie sich bitte an Ihre nächstgelegene Sysmex Vertretung.

7 Vorsichtsmaßnahmen und Warnhinweise
Die Reagenzien enthalten 14 mM Natriumazid als Konservierungsstoff. Aufgrund der niedrigen Natriumazid Konzentration ist eine Gefahrstoffkennzeichnung nicht erforderlich, jedoch sind die beim Umgang mit Chemikalien üblichen Vorsichtsmaßregeln zu beachten. Beachten Sie das Sicherheitsdatenblatt (SDB) für eine vollständige Liste von Warnhinweise und Vorsichtsmaßnahmen.

8 Zusätzlich erforderliche Ausrüstung

Erforderliche Instrumente:	CyFlow™ Counter (REF No.: CY-S-3022)
Benötigte Ausrüstung:	Probenröhrchen 3.5 ml (Code No.: 04-2000) Eine verifizierte 10-µl-Pipette und Pipettenspitzen Eine verifizierte 20-µl-Pipette und Pipettenspitzen Eine verifizierte 100- bis 1000-µl-Pipette und Pipettenspitzen System zur venösen Blutentnahme mit EDTA als Antikoagulans Stoppuhr

9 Reagent preparation
CD4 mAb PE (REF No.: 05-8405-01), das Antikörperreagens ist gebrauchsfertig.
CD45 mAb PE-Cy5 (REF No.: 05-8405-02), das Antikörperreagens ist gebrauchsfertig.
Puffer 1 (REF No.: 05-8405-03), die Pufferlösung ist gebrauchsfertig.
Puffer 2 (REF No.: 05-8405-04), die Pufferlösung ist gebrauchsfertig.

10 Entnahme, Handhabung und Lagerung der Primärprobe
WARNUNG ⚠ *Alle biologischen Proben und Materialien sind als gefährliches biologisches Material und als potenziell infektiös zu behandeln. Es sind angemessene Sicherheitsvorkehrungen und Verfahren zur Handhabung gemäß den geltenden Gesetzen und Vorschriften anzuwenden.*

- Bei der Blutprobe muss es sich um eine venöse Vollblut-Probe handeln, die mithilfe eines Blutentnahmesystems entnommen wurde, das EDTA als Antikoagulans verwendet.
- Die Blutprobe ist in einem dunklen, vor Licht geschützten Behälter zu transportieren und darf nicht erhöhten Temperaturen ausgesetzt werden. Ein geeigneter Transportbehälter ist z. B. eine Styroporbox mit Kühlung. Die besten Transportbedingungen würden unter 25 °C liegen, wenn der Transport nicht länger als 6 Stunden nach der Spende dauert.
- Für eine Transport- und Lagerzeit von mehr als 6 Stunden muss das Blut bei 2 - 8 °C aufbewahrt werden
- Die Blutprobe nicht einfrieren und wieder auftauen.
- Optimale Bedingungen sind bei Verwendung einer frischen Blutprobe gegeben, d. h. es sollten nicht mehr als sechs Stunden zwischen der Blutentnahme und der Analyse liegen.
- Bei Lagerung im Kühlschrank bei 2 - 8 °C können Blutproben bis zu 24 Stunden nach der Entnahme verwendet werden.
- Vor der Anwendung die Probenröhrchen 8 - 10 mal vorsichtig umdrehen.
- Keine geronnenen Blutproben verwenden.

11 Untersuchungsverfahren
HINWEIS *Reverses Pipettieren ist entscheidend für die Genauigkeit, insbesondere bei der Abgabe viskoser und sehr kleiner Probenmengen. Für das Pipettieren von Vollblut empfehlen wir die Verwendung einer kalibrierten elektronischen Pipette, die für den Betrieb im reversen Pipettiermodus vorprogrammiert ist.*
Steht keine elektronische Pipette zur Verfügung, führen Sie das reverse Pipettieren gemäß den folgenden Anweisungen manuell durch:
- *Bedienknopf bis zum zweiten Anschlag herunterdrücken. Bedienknopf vollständig zurückgleiten lassen. Es wird eine größere Menge Probe in die Spitze aufgezogen.*
- *Bedienknopf bis zum ersten Anschlag herunterdrücken, um ein genaues Blutvolumen abzugeben. Anschließend befindet sich noch ein Rest der Probe in der Spitze.*
- *Pipettenspitze mit dem verbliebenen Rest der Blutprobe in einen Behälter für medizinische Abfälle entsorgen.*

Beachten Sie bitte, dass reverses Pipettieren nicht für Antikörper- oder Pufferlösungen eingesetzt werden darf.

11.1 Markierung
- Die Blutprobe im EDTA-Blutentnahmeröhrchen 8 - 10 mal vorsichtig umdrehen.
- 20 µl EDTA-Vollblutprobe auf den Boden eines Probenröhrchens pipettieren. Dabei keine Blutspuren von der Pipettenspitze auf der Röhrcheninnenwand hinterlassen. Pipettenspitze entsorgen.
- 10 µl CD4 mAb PE direkt in die Blutprobe geben und vorsichtig mischen. Dabei keine Blutspuren von der Pipettenspitze auf der Röhrcheninnenwand hinterlassen. Pipettenspitze entsorgen.
- 10 µl CD45 mAb PE-Cy5 direkt in die Blutprobe geben und vorsichtig mischen. Dabei keine Blutspuren von der Pipettenspitze auf der Röhrcheninnenwand hinterlassen. Pipettenspitze entsorgen.
- Inkubieren Sie die Mischung 15 Minuten bei 15 - 30 °C im Dunkeln.
- 400 µl Puffer 1 hinzugeben und kurz vortexen oder das Röhrchen sanft aufklopfen, um die Probe zu mischen. Pipettenspitze entsorgen.
- 400 µl Puffer 2 hinzugeben und kurz vortexen oder das Röhrchen sanft aufklopfen, um die Probe zu mischen. Pipettenspitze entsorgen.
- Vor der Analyse mit dem CyFlow™ Counter kurz vortexen oder das Röhrchen sanft aufklopfen.

HINWEIS *Nach der Zugabe von Puffer 1 können die Proben bis zu 2 Stunden bei 2 - 8 °C im Dunkeln aufbewahrt werden. Die Probe danach nicht mehr verwenden und auch dann nicht, wenn sie nicht wie angegeben vorbereitet wurde.*
Nach der Zugabe von Puffer 2 müssen die Proben innerhalb von 10 Minuten analysiert werden.

11.2 Probenanalyse
Bitte vor der Probenanalyse das Benutzerhandbuch des CyFlow™ Counter (CY-S-3022IFUEN) für die Verwendung des Geräts beachten. Vor der Probenanalyse müssen die Verfahren zur Inbetriebnahme und internen Qualitätskontrolle erfolgreich abgeschlossen worden sein.
- Wählen und laden Sie die Konfiguration für die CD4% Messung aus der Menüleiste des CyFlow™ Counter.
- Das Probenröhrchen mit dem aufbereiteten Blut in den Probenport setzen.
- Die Messung starten.
- Nach dem Probendurchlauf stoppt das Instrument und wird automatisch gereinigt.
- Die Blutprobe entnehmen und gemäß den örtlichen Sicherheitsvorschriften in Bezug auf biogefährliche Materialien in Labors entsorgen.

Keine erneute Messung mit demselben Probenröhrchen durchführen. Wenn eine Zweifachmessung oder eine Wiederholung der Messung erforderlich ist, muss ein zweites Probenröhrchen wie unter 11.1 beschrieben vorbereitet werden.

11.3 Datenerfassung und -analyse
Die Datenanalyse kann erst erfolgen, wenn die interne Qualitätskontrolle des CyFlow™ Counter erfolgreich abgeschlossen wurde. Im Benutzerhandbuch des CyFlow™ Counter (CY-S-3022IFUEN) finden Sie eine Beschreibung der Inbetriebnahme und Wartung des Geräts. Das CD4-T-Zell-Zählresultat, der CD4-Prozentsatz und die Gesamtzahl der Lymphozyten werden auf dem Bildschirm angezeigt. Wenn die Haufen der markierten Zellen im CD45 SSC-Dot Plot von Rauschsignalen nicht eindeutig abgrenzbar sind (siehe Abbildung 2) oder die Haufen CD4-positiver Zellen, CD4-negativer Zellen und Monozytenhaufen im CD4 SSC-Dot Plot nicht eindeutig voneinander abgrenzbar sind, muss die Probenvorbereitung und -messung wiederholt werden.

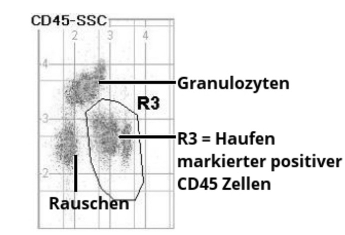


Abbildung 1: Beispiel für gute Abgrenzung von Granulozyten-Haufen, anderen markierten CD45-positiven Leukozyten und Rauschsignalen.

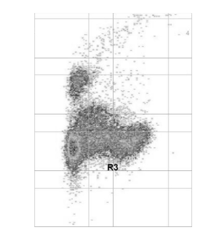


Abbildung 2: Beispiel für eine schlechte Trennung zwischen Rauschen und CD45-positiv gefärbten Leukozyten. Diese Probe ist nicht analysierbar.

Es kommt zu falschen Ergebnissen, wenn die Positionierung von CD45-positiv gefärbten Zellen (R3) nicht genau erfolgt.

12 Berechnung der Untersuchungsergebnisse
Die Berechnung der Zellkonzentration erfolgt im Rahmen der Software-basierten Datenanalyse. Die Ergebnisse werden nach jeder Messung als CD4%, CD4+-Zellen pro µl Blutprobe und Lymphozyten pro µl Blutprobe dargestellt.
Weitere Informationen entnehmen Sie bitte dem Benutzerhandbuch des CyFlow™ Counter (CY-S-3022IFU-EN).

13 Interpretation der Ergebnisse
Das Humane Immundefizienzvirus (HIV) ist einer der Hauptgründe für die CD4-Zelldepletion, die das Immunsystem schwächt. Die Ergebnisse sollten von Ärzten in Verbindung mit lokal anwendbaren HIV-Behandlungsrichtlinien interpretiert werden.

14 Kontrollverfahren
Es ist eine geeignete Qualitätskontrolle gemäß den einschlägigen lokalen und nationalen Vorschriften durchzuführen. Im Handel sind unterschiedliche Arten von stabilisierten Kontrollblutproben erhältlich. Nicht alle sind für jedes Flowzytometer geeignet.Welches Kontrollblut Sie verwenden können, erfahren Sie bei Ihrer Sysmex-Vertretung. Sind nach der Messung einer geeigneten Kontrollblutprobe die Akzeptanzkriterien nicht erfüllt, fahren Sie nicht fort mit der Analyse von Patientenproben.

15 Leistungsmerkmale
15.1 Spezifität
Der MEM-241-Antikörper erkennt das CD4-Antigen, ein transmembranes Glykoprotein mit einem Molekulargewicht von 55 kDa, das auf einer Untergruppe von T-Lymphozyten (T-Helferzellen) exprimiert wird, sowie auf Monozyten, Gewebsmakrophagen und Granulozyten. HCDM (ehemals HLDA VIII) Meeting, Mai 2006, Québec, Kanada; WS Code M241.
Der Antikörper MEM-28 reagiert mit allen Isoformen des humanen CD45-Antigens (Leukocyte Common Antigen), ein transmembranes Typ-I-Einzelkettenprotein mit einem Molekulargewicht von 180-220 kDa, das in hohem Maße auf allen Zellen hämatopoetischen Ursprungs, mit Ausnahme von Erythrozyten und Thrombozyten, exprimiert wird. HLDA III; WS Code NL 833a.

15.2 Nachweisfähigkeit
Die Leerwert-Obergrenze (Limit of Blank, LoB), die Nachweisgrenze (Limit of Detection, LoD) und die Quantifizierungsgrenze (Limit of Quantitation, LoQ) wurden gemäß den Spezifikationen der Richtlinie CLSI EP17-A2 ermittelt.
LoB: ≤50% of LoD
LoD: ≤20 CD4 Zellen/µl
LoQ: ≤40 CD4 Zellen/µl

15.3 Reproduzierbarkeit
Intra- und Inter-Assay-Varianz:
Die CV-Werte für CD4-Werte ≥ 200 CD4-T-Zellen/µl liegen unter 10 %.
Die CV-Werte für CD4%-Werte ≥ 25% liegen unter 10 %.
Die CV-Werte für absolute CD4-Werte < 200 CD4-T-Zellen/µl liegen unter 15 %.
Die Werte der Standardabweichung (SD) ≤ 2.5% für CD4% Werte liegen unter 25%.

15.4 Linearität
Der Messbereich des Assay und die Linearität der Messungen gelten für den folgenden Bereich:
Absoluter CD4 Wert: 40-2500 CD4 T-Zellen/µl
CD4%: 4 – 60%

15.5 Richtigkeit
EDTA-Vollblutproben wurden mit dem CD4% easy count kit markiert und im CyFlow™ Counter (CyView™ 2.11) analysiert. Die absoluten CD4-Werte und die CD4%-Werte wurden mit Ergebnissen eines BD FACSCalibur unter Verwendung von BD Tritest CD3/CD4/CD45 und BD Trucount-Röhrchen verglichen. Es zeigte sich eine Abweichung von -22,56 % (CD4 absolut) und +2,1 % (CD4%) zwischen den Methoden.

16 Grenzen des Verfahrens
ACHTUNG ⚠ *Die Sysmex Partec CD4% easy count kits (05-8405) wurden nicht für die Verwendung bei Kindern und Jugendlichen validiert.*

ACHTUNG ⚠ *Patientenmessungen von CD4 und CD4% mithilfe von Sysmex Partec Assays sollten nicht zusammen mit Methoden anderer Hersteller zur Bestimmung von CD4 und CD4% verwendet werden. Verwenden Sie die Assays nur mit Sysmex Partec Geräten. Werte, die mit anderen Geräten oder Assays erhalten wurden, sind nicht austauschbar.*

Verschiedene endogene und exogene Substanzen wurden in Übereinstimmung mit der Richtlinie CLSI EP07-A2 auf eine mögliche Beeinträchtigung der Blutprobenanalyse untersucht. Bei ≥ 18 % Hämolyse (gemäß dem in der Richtlinie CLSI EP07-A2, Anhang G empfohlenen Verfahren G1 „Osmotic Shock Procedure" induziert) wurde eine Beeinträchtigung beobachtet. Aus diesem Grund sollten hämolytische Proben verworfen werden. Für die Bestimmung von CD4 und CD4% in Vollblutproben mit CyFlow™ Counter Assays sollte kein anderes Antikoagulans als EDTA verwendet werden. Bei weiteren Fragen hinsichtlich des Leistung des Produkts wenden Sie sich bitte an Ihre nächstgelegene Sysmex-Vertretung.

17 Literatur
1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondière, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
2) L. Zijenah, G. Kadzirange, S. Madzime, M. Borok, C. Mudiwa, O. Tobaiwa, M. Mucheche, S. Rusakaniko, D. Katzenstein. (2006). Affordable flow cytometry for enumeration of absolute CD4+ T-lymphocytes to identify subtype C HIV-I infected adults requiring antiretroviral therapy (ART) and monitoring response to ART in a resource-limited setting. Journal of Translational Medicine 4:33
3) H. Karcher, D. Bohning, R. Downing, S. Mashate, G. Harms. (2006). Comparison of two alternative methods for CD4+ T-cell determination (Coulter manual CD4 count and CyFlow) against standard dual platform flow cytometry in Uganda. Clinical Cytometry, 70B (3):163-69
4) S. Kohreanudom, S. Dettraira. (2012). Evaluation of the Partec Single-Platform Volumetric CyFlow® Counter System for Determining Percentage and Absolute Numbers of CD4 T Lymphocytes in HIV/AIDS Thai Patients. Thai AIDS J, 25:1-10
5) D. Wade, P.A. Diaw, G. Daneau, M. Camara, T.N. Dieye, S. Mboup, L. Kestens. (2013). CD4 T-Cell Enumeration in a Field Setting: Evaluation of CyFlow Counter Using the CD4 Easy Count Kit-Dry and Pima CD4 Systems. PLoS ONE, 8(9): e75484

18 Kontaktinformationen des Herstellers
Manufacturer
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Deutschland
www.sysmex-partec.com
Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Versionsnummer des Benutzerhandbuchs und Ausgabedatum
Revision: Rev-07_10-09-2020_CN 1563
Issued by: Sysmex Partec GmbH

Das Sicherheitsdatenblatt zu diesem Produkt ist unter www.sysmex-partec.com/services erhältlich.

Identification du réactif IVD
Nom du produit :
CD4% easy count kit
REF 05-8405

Contenu:

Fiole de CD4 mAb PE	REF 05-8405-01
Fiole de CD45 mAb PE-Cy5	REF 05-8405-02
Flacon de tampon 1	REF 05-8405-03
Flacon de tampon 2	REF 05-8405-04

1 Spécification

Spécificité	CD4 humain	CD45 humain
Isotype	IgG1	IgG1
Contenu	100 tests	100 tests
Fluorochrome	PE	PE-Cy5
Excitation λ (nm)	532 / 488	532 / 488
Émission maximale(nm)	578	670

2 Usage prévu
IVD Pour la réalisation de diagnostics in vitro.

Le CD4% easy count kit est un test IVD quantitatif manuel constitué de quatre composants et destiné au marquage des leucocytes et d'une sous-population de lymphocytes dans le sang veineux total EDTA chez l'adulte, pouvant ensuite être dénombrés à l'aide du cytomètre en flux IVD CyFlow™ Counter de Sysmex Partec. La concentration en cellules T CD4 et le taux CD4% de lymphocytes dans les échantillons de sang sont des indicateurs utile pour l'initiation ou le suivi du traitement des porteurs du VIH, conjointement avec d'autres résultats cliniques et de laboratoire.
Le test doit être effectué par des professionnels de santé formés à cet effet.

3 Principe de la méthode d'examen
Une partie aliquote d'un échantillon de sang total EDTA est mélangée à deux anticorps (CD4 et CD45), conjugués chacun à un fluorochrome différent pour le marquage de populations cellulaires spécifiques. Après un temps d'incubation déterminé, les deux solutions tampons sont ajoutées et l'échantillon est prêt pour l'analyse sur un cytomètre en flux CyFlow™ Counter. La source de lumière excite le colorant fluorescent lié à la cellule colorée et la lumière émise est détectée alors qu'un volume précis d'échantillon de sang traverse l'instrument. Le logiciel intégré calcule la concentration des populations cellulaires spécifiques.
Pour de plus amples informations, se reporter au guide d'utilisation du CyFlow™ Counter (CY-S-3022IFU-EN).

4 Stockage et durée de conservation après la première ouverture
1. Stockage:
Stocker l'anticorps et les réactifs tampons à une température comprise entre 2 - 8 °C à l'abri de la lumière. Ne pas congeler ni exposer les réactifs à des températures élevées et les protéger du rayonnement solaire direct.
2. Durée de conservation après la première ouverture:
Toujours fermer le flacon après usage et utiliser de nouveaux embouts de pipettes à chaque fois qu'un échantillon de réactif est prélevé pour éviter toute contamination.
Dans ces conditions de stockage, le CD4% easy count kit restera stable jusqu'à la date de péremption imprimée sur l'étiquette du kit.

5 Composants
Le CD4 mAb PE et le CD45 mAb PE-Cy5 sont des anticorps monoclonaux murins fournis dans un tampon PBS avec 0,2 % de BSA et 0,09 % d'azoture de sodium. Le tampon 1 et le tampon 2 sont des solutions à base de PBS contenant 0,09 % d'azoture de sodium.

6 Signes de détérioration
La solution d'anticorps et la solution tampon sont des liquides clairs. Ne pas utiliser les réactifs en cas d'apparition d'un quelconque signe de turbidité ou de contamination. Pour toutes questions concernant la performance ou la qualité du produit, veuillez contacter votre représentant Sysmex local pour l'assistance technique.

7 Avertissements et mesures de précaution
Les réactifs contiennent de l'azoture de sodium 14 mM comme agent conservateur. La faible concentration de l'azoture de sodium ne nécessite aucun marquage comme substance dangereuse, mais les mesures de sécurité normales relatives à la manipulation des produits chimiques doivent être observées. Se reporter à la fiche de données de sécurité pour obtenir la liste complète des avertissements et mesures de précaution.

8 Équipement supplémentaire requis

Instruments requis:	CyFlow™ Counter (REF No.: CY-S-3022)
Matériel requis:	Tubes d'échantillon 3.5 ml (REF No.: 04-2000) Une pipette 10 µl validée et embouts de pipette Une pipette 20 µl validée et embouts de pipette Une pipette 100 – 1000 µl validée et embouts de pipette Système de prélèvement de sang veineux avec EDTA comme anticoagulant Chronomètre

9 Préparation des réactifs
CD4 mAb PE (REF No.: 05-8405-01), le réactif à base d'anticorps est prêt à l'emploi.
CD45 mAb PE-Cy5 (REF No.: 05-8405-02), le réactif à base d'anticorps est prêt à l'emploi.
Tampon 1 (REF No.: 05-8405-03), la solution tampon est prête à l'emploi.
Tampon 2 (REF No.: 05-8405-04), la solution tampon est prête à l'emploi.

10 Prélèvement, manipulation et stockage de l'échantillon primaire
AVERTISSEMENT ⚠ *Tous les échantillons et matériaux biologiques sont considérés comme biologiquement dangereux et doivent être manipulés comme s'ils étaient susceptibles de transmettre une infection. Les mesures de sécurité et les procédures de manipulation appropriées doivent être appliquées conformément aux lois et aux réglementations en vigueur.*

- L'échantillon de sang doit être un échantillon de sang veineux total, prélevé à l'aide d'un système de prélèvement sanguin contenant de l'EDTA comme anticoagulant.
- L'échantillon de sang doit être transporté dans un récipient sombre, hermétique à la lumière, et ne doit pas être exposé à des températures élevées. Une boîte en polystyrène avec un dispositif de réfrigération, par exemple, constitue un conteneur de transport approprié. Les meilleures conditions de transport seraient en dessous de 25 °C si le transport ne prend pas plus de 6 heures après le don.
- Pour le transport et le stockage plus de 6 heures, le sang doit être conservé entre 2 - 8 °C.
- Ne pas congeler ni décongeler l'échantillon de sang.
- Pour des conditions optimales, l'échantillon de sang doit être frais, c'est-à-dire qu'il ne doit pas s'être écoulé plus de six heures entre le prélèvement de l'échantillon et l'analyse.
- Les échantillons de sang peuvent être utilisés jusqu'à 24 heures après la prise de sang, s'ils sont conservés au réfrigérateur à une température comprise entre 2 - 8 °C.
- Avant l'utilisation, les tubes d'échantillon de sang doivent être retournés doucement 8 - 10 fois.
- Ne pas utiliser des échantillons de sang coagulé.

11 Procédure d'examen
REMARQUE *Le pipettage à l'envers est essentiel à la précision, tout particulièrement en cas de délivrance d'échantillons visqueux et de volumes d'échantillons très réduits. Pour le pipettage de sang total, nous recommandons l'utilisation d'une pipette électronique calibrée qui est préprogrammée pour fonctionner en mode de pipettage à l'envers.*
En l'absence d'une pipette électronique, suivre les présentes consignes pour procéder à un pipettage manuel à l'envers :
- *Enfoncer le bouton d'actionnement jusqu'à la deuxième butée. Laisser le bouton remonter entièrement, l'excès d'échantillon est aspiré dans la pointe.*
- *Enfoncer le bouton d'actionnement jusqu'à la première butée pour expulser un volume précis de sang tout en conservant l'excès d'échantillon dans la pointe.*
- *Éliminer la pointe de pipette contenant l'excès d'échantillon de sang dans une boîte à déchets médicaux.*

Prière de noter qu'il n'est pas nécessaire de procéder au pipettage à l'envers pour les anticorps ou les solutions tampons.

11.1 Marquage
- Renverser doucement l'échantillon de sang dans le tube de prélèvement de sang EDTA 8 - 10 fois.
- Pipeter 20 µl d'échantillon de sang total EDTA dans le fond d'un tube d'échantillon, en évitant de laisser un filet de sang sur la paroi intérieure du tube avec l'embout de pipette. Jetez l'embout de la pipette.
- Ajouter 10 µl de CD4 mAb PE directement dans l'échantillon de sang et mélanger doucement en évitant de laisser un filet de sang sur la paroi intérieure du tube avec l'embout de pipette. Jetez l'embout de la pipette.
- Ajouter 10 µl de CD45 mAb PE-Cy5 directement dans l'échantillon de sang et mélanger doucement en évitant de laisser un filet de sang sur la paroi intérieure du tube avec l'embout de pipette. Jetez l'embout de la pipette.
- Incuber le mélange pendant 15 minutes entre 15 - 30 °C et à l'abri de la lumière.
- Ajouter 400 µl de tampon 1 et mélanger brièvement au vortex ou tapoter doucement le tube afin de mélanger l'échantillon. Jetez l'embout de la pipette.
- Ajouter 400 µl de tampon 2 et mélanger brièvement au vortex ou tapoter doucement le tube afin de mélanger l'échantillon. Jetez l'embout de la pipette.
- Mélanger brièvement au vortex ou tapoter doucement le tube avant d'effectuer l'analyse à l'aide du CyFlow™ Counter.

REMARQUE *Après l'ajout du tampon 1, il est possible de conserver les échantillons jusqu'à 2 heures à une température comprise entre 2 - 8 °C à l'abri de la lumière. Ne pas utiliser l'échantillon après ce délai ou s'il n'a pas été préparé de manière conforme.*
Les échantillons doivent être analysés dans un délai de 10 minutes après l'ajout du tampon 2.

11.2 Analyse de l'échantillon
Pour obtenir des informations sur le fonctionnement de l'instrument avant l'analyse de l'échantillon, se reporter au guide d'utilisation du CyFlow™ Counter (CY-S-3022IFUEN). Les procédures de démarrage et la procédure de contrôle qualité interne doivent avoir été effectuées avec succès avant l'analyse de l'échantillon.
- Choisissez et chargez la configuration pour la mesure CD4% dans la barre de menu de CyFlow™ Counter.
- Insérer le tube d'échantillon avec l'échantillon de sang préparé dans l'orifice d'échantillonnage.
- Démarrer la mesure.
- Après l'analyse de l'échantillon, l'instrument s'arrête et se nettoie automatiquement.
- Retirer et éliminer l'échantillon de sang conformément aux procédures locales de sécurité en laboratoire relatives aux risques biologiques.

Ne pas démarrer de seconde mesure avec le même tube d'échantillon. S'il faut répéter ou effectuer deux fois la mesure, préparer un second tube d'échantillon conformément au point 11.1.

11.3 Acquisition et analyse des données
L'analyse des données à l'aide du CyFlow™ Counter n'est possible que si le contrôle qualité interne de l'instrument a été effectué avec succès. Consulter le guide d'utilisation du CyFlow™ Counter (CY-S-3022IFU-EN) pour effectuer une procédure de démarrage et la maintenance de l'appareil, si nécessaire.
Le résultat du comptage des cellules T CD4, le taux de pourcentage CD4 et la quantité totale de lymphocytes s'affichent à l'écran. Si les amas de cellules marquées ne sont pas clairement différenciables des signaux de bruit de fond dans le dot plot CD45 – SSC (voir Figure 2), ou si les amas de cellules positives CD4, de cellules négatives CD4 et de monocytes ne sont pas clairement différenciables dans le dot plot CD4 – SSC, il faut recommencer la préparation et la mesure de l'échantillon.

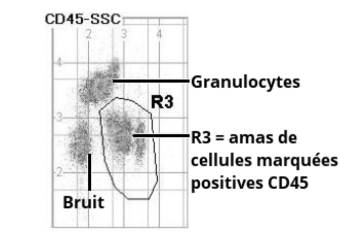


Figure 1: Exemple de bonne différenciation des amas de granulocytes, d'autres leucocytes marqués positifs CD45 et des signaux de bruit.

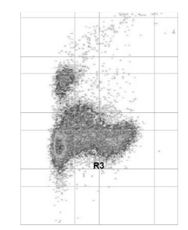


Figure 2: Exemple de mauvaise séparation entre les signaux de bruit et les leucocytes colorés CD45. Cet échantillon n'est pas analysable.

Les résultats seront incorrects si le positionnement de la porte des cellules colorées positives CD45 (R3) n'est pas fait avec précision.

12 Calcul des résultats d'examen
Le calcul de la concentration cellulaire fait partie intégrante de l'analyse des données par logiciel qui fournit le résultat en CD4%, en cellules CD4+ par µl d'échantillon de sang et en lymphocytes par µl d'échantillon de sang, après chaque mesure.
Pour de plus amples informations, se reporter au guide d'utilisation du CyFlow™ Counter (CY-S-3022IFUEN).

13 Interprétation des résultats
Le virus d'immunodéficience humaine (VIH) est l'une des principales raisons de la déplétion des cellules CD4, ce qui affaiblit le système immunitaire. Les résultats doivent être interprétés par les médecins, en conjonction avec les directives de traitement du VIH applicables localement.

14 Procédure de contrôle
Un contrôle qualité approprié doit être effectué conformément aux réglementations locales et nationales. Il existe différents types d'échantillons de sang de contrôle stabilisé disponibles sur le marché de détail. Tous ne conviennent pas à chaque cytomètre en flux. Vous pouvez trouver le sang de contrôle que vous utilisez à votre représentant Sysmex. Si les critères d'acceptation ne sont pas satisfaits après la mesure d'un échantillon de sang de contrôle approprié, ne pas procéder à l'analyse des échantillons de patients.

15 Caractéristiques de performance
15.1 Spécificité
L'anticorps MEM-241 reconnaît l'antigène CD4, une glycoprotéine transmembranaire 55 kDa exprimée sur une sous-population de lymphocytes T (cellules T « auxiliaires »), ainsi que sur les monocytes, les macrophages tissulaires et les granulocytes. HCDM (former HLDA VIII) Meeting, mai 2006, Québec, Canada ; WS Code M241
L'anticorps MEM-28 réagit avec toutes les formes alternatives de l'antigène CD45 humain (Leukocyte Common Antigen), une protéine transmembranaire à chaîne unique de type I 180-220 kDa exprimée en forte densité sur toutes les cellules d'origine hématopoïétique, à l'exception des érythrocytes et des plaquettes. HLDA III ; WS Code NL 833a

15.2 Capacité de détection
Les évaluations de la limite du blanc (LoB), de la limite de détection (LoD) et de la limite de quantification (LoQ) ont été réalisées conformément aux spécifications de la directive CLSI EP17-A2.
LoB : ≤ 50 % de la LoD
LoD : ≤ 20 cellules CD4/µl
LoQ : ≤ 40 cellules CD4/µl

15.3 Répétabilité
Variation intra- et inter-essai :
Les valeurs du coefficient de variation (CV) sont inférieures à 10 % pour des valeurs de CD4 supérieures ou égales à 200 cellules T CD4/µl.
Les valeurs du coefficient de variation (CV) sont inférieures à 10 % pour des valeurs de CD4% supérieures ou égales à 25 %.
Les valeurs du coefficient de variation (CV) sont inférieures à 15 % pour des valeurs absolues de CD4 inférieures à 200 cellules T CD4/µl.
Les valeurs de l'écart-type (SD) ≤ 2,5% pour les valeurs CD4% sont inférieures à 25%.

15.4 Linéarité
La plage de mesure de l'essai et la linéarité de la mesure sont valides sur la plage :
taux absolu de CD4 : 40-2500 cellules T CD4/µl
CD4% : 4 – 60%

15.5 Justesse
Les échantillons de sang total EDTA ont été marqués avec le CD4% easy count kit et analysés sur le CyFlow™ Counter (CyView™ 2.11). Les valeurs absolues de CD4 et les valeurs de CD4% ont été comparées aux résultats obtenus à l'aide d'un BD FACSCalibur en utilisant des réactifs BD Tritest CD3/CD4/CD45 et des tubes BD Trucount, ce qui a révélé des écarts de -22,56 % (CD4 absolu) et +2,1 % (CD4%) entre les deux méthodes.

16 Limites de la procédure
PRUDENCE ⚠ *L'utilisation des kits CD4% easy count (05-8405) Sysmex Partec n'a pas été validée chez les patients pédiatriques et adolescents.*

PRUDENCE ⚠ *Les mesures des patients de CD4 et de CD4% effectuées à l'aide des tests Sysmex Partec ne doivent pas être utilisées avec les mesures obtenues à partir des méthodes de détermination des CD4 et CD4% d'autres fabricants. Utilisez les tests uniquement avec les instruments Sysmex Partec. Les valeurs obtenues avec d'autres équipments ou dosages ne sont pas interchangeables.*

Différentes substances endogènes et exogènes ont été testées conformément à la directive CLSI EP07-A2 pour vérifier les éventuelles interférences pouvant avoir une influence sur l'analyse de l'échantillon de sang. Il a finalement été observé ≥ 18 % d'hémolyse (engendrée par l'utilisation du protocole recommandé dans la directive CLSI EP07-A2, annexe G, G1 – Procédure de choc osmotique) provoquant des interférences. C'est pourquoi les échantillons hémolysés devrait être écartés. Pour la détermination des CD4 et CD4% dans les échantillons de sang total avec des dosages CyFlow™ Counter, aucun autre anticoagulant que l'EDTA ne doit être utilisé. Pour toutes questions supplémentaires concernant la performance du produit, veuillez contacter votre représentant Sysmex local.

17 Bibliographie
1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondière, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
2) L. Zijenah, G. Kadzirange, S. Madzime, M. Borok, C. Mudiwa, O. Tobaiwa, M. Mucheche, S. Rusakaniko, D. Katzenstein. (2006). Affordable flow cytometry for enumeration of absolute CD4+ T-lymphocytes to identify subtype C HIV-I infected adults requiring antiretroviral therapy (ART) and monitoring response to ART in a resource-limited setting. Journal of Translational Medicine 4:33
3) H. Karcher, D. Bohning, R. Downing, S. Mashate, G. Harms. (2006). Comparison of two alternative methods for CD4+ T-cell determination (Coulter manual CD4 count and CyFlow) against standard dual platform flow cytometry in Uganda. Clinical Cytometry, 70B (3):163-69
4) S. Kohreanudom, S. Dettraira. (2012). Evaluation of the Partec Single-Platform Volumetric CyFlow® Counter System for Determining Percentage and Absolute Numbers of CD4 T Lymphocytes in HIV/AIDS Thai Patients. Thai AIDS J, 25:1-10
5) D. Wade, P.A. Diaw, G. Daneau, M. Camara, T.N. Dieye, S. Mboup, L. Kestens. (2013). CD4 T-Cell Enumeration in a Field Setting: Evaluation of CyFlow Counter Using the CD4 Easy Count Kit-Dry and Pima CD4 Systems. PLoS ONE, 8(9): e75484

18 Coordonnées du fabricant
Fabricant
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Allemagne
www.sysmex-partec.com
Tél +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Numéro de version du guide d'utilisation et date de publication
Révision: Rev-07_10-09-2020_CN 1563
Publiée par: Sysmex Partec GmbH

La fiche de données de sécurité de ce produit est disponible à l'adresse suivante : www.sysmex-partec.com/services.

Instrucciones de uso

Identificación del reactivo para DIV
Nombre del producto:
Kit CD4% easy count   REF 05-8405

Contenido::
Vial con CD4 mAb PE   REF 05-8405-01
Vial con CD45 mAb PE-Cy5   REF 05-8405-02
Botella con solución amortiguadora 1   REF 05-8405-03
Botella con solución amortiguadora 2   REF 05-8405-04

1 Descripción

Especificidad	CD4 humano	CD45 humano
Isotipo	IgG1	IgG1
Contenido	100 tests	100 tests
Fluorocromo	PE	PE-Cy5
λ de excitación (nm)	532 / 488	532 / 488
Emisión máxima (nm)	578	670

2 Uso previsto
IVD Para diagnóstico in vitro.
El kit CD4% easy count es un test cuantitativo manual de DIV de cuatro componentes para el marcaje de leucocitos y una subpoblación de linfocitos en sangre entera venosa adulta con EDTA, que pueden a continuación enumerarse con el citómetro de flujo para DIV CyFlow™ Counter de Sysmex Partec. La concentración de linfocitos T CD4 y el CD4% de linfocitos da las muestras de sangre son indicadores útil para iniciar o supervisar el tratamiento de personas inectadas por el VIH junto con otros hallazgos clínicos y de laboratorio.
El test está previsto para su uso por parte de profesionales sanitarios con la formación correspondiente.

3 Principio del método de análisis
Se mezcla una cantidad equivalente de una muestra de sangre entera con EDTA con dos anticuerpos (CD4 y CD45), cada uno conjugado con un fluorocromo diferente para marcar poblaciones celulares específicas. Tras un tiempo fijo de incubación, se añaden las dos soluciones amortiguadoras y la muestra está lista para su análisis en el citómetro de flujo CyFlow™ Counter. La fuente de luz excita el tinte fluorescente vinculado con la célula teñida y la luz emitida se detecta mientras un volumen preciso de muestra de sangre pasa por el instrumento. La concentración de poblaciones celulares específicas se calcula con el software integrado. Para más información, consulte las instrucciones de uso de CyFlow™ Counter (CY-S-3022IFUEN).

4 Almacenamiento y tiempo de conservación tras abrir por primera vez
1. Almacenamiento:
Almacene los reactivos de anticuerpos y solución amortiguadora en un lugar oscuro a 2 - 8 °C. No congele ni exponga los reactivos a temperaturas elevadas o a la luz solar directa.
2. Tiempo de conservación tras abrir por primera vez:
Cierre siempre la botella después de su uso y utilice puntas de pipeta nuevas cada vez que tome muestras del reactivo para evitar la contaminación.
En estas condiciones de almacenamiento, el kit CD4% easy count permanecerá estable hasta la fecha de caducidad impresa en la etiqueta del kit.

5 Componentes
mAb CD4-PE y mAb CD45-PE-Cy5 son anticuerpos monoclonales murinos suministrados en una solución amortiguadora PBS con un 0,2% de SAB y un 0,09% de azida de sodio. Las soluciones amortiguadoras 1 y 2 son soluciones con base de PBS que contienen un 0,09% de azida de sodio.

6 Indicios de deterioro
El reactivo de anticuerpos y la solución amortiguadora son líquidos transparentes. No utilice el reactivo si aparece cualquier tipo de turbidez o contaminación. Si tiene alguna pregunta con respecto al funcionamiento o la calidad del producto, contacte con su representante local de Sysmex y solicite asistencia técnica.

7 Precaución y advertencias
Los reactivos contienen 14 mM de azida de sodio como conservante. Dada su reducida concentración de azida de sodio, no requiere un etiquetado como producto de riesgo, pero se deben observar las precauciones de seguridad normales para la manipulación de sustancias químicas. Consulte la ficha técnica de seguridad para obtener una lista completa de advertencias y precauciones.

8 Equipamiento adicional necesario

Instrumental necesario:	CyFlow™ Counter (REF No.: CY-S-3022)
Equipamiento requerido:	Tubos de ensayo de 3.5 ml (REF No.: 04-2000) Una pipeta verificada de 10 µl y puntas de pipeta Una pipeta verificada de 20 µl y puntas de pipeta Una pipeta verificada de 100 – 1000 µl y puntas de pipeta Sistema de extracción de sangre venosa con EDTA como anticoagulante Cronómetro

9 Preparación del reactivo
CD4 mAb PE (REF No.: 05-8405-01), el reactivo de anticuerpos está listo para su uso.
CD45 mAb PE-Cy5 (REF No.: 05-8405-02), el reactivo de anticuerpos está listo para su uso.
Solución amortiguadora 1 (REF No.: 05-8405-03), la solución amortiguadora está lista para su uso.
Solución amortiguadora 2 (REF No.: 05-8405-04), la solución amortiguadora está lista para su uso.

10 Principios básicos de extracción, manipulación y almacenamiento de muestras

ADVERTENCIA ⚠ *Todas las muestras y materiales biológicos se consideran material de riesgo biológico y deberían manipularse como si fueran potencialmente infecciosos. Se deben observar las precauciones de seguridad y procedimientos de manipulación adecuados conforme a la legislación y normativa.*

- La muestra de sangre debe ser una muestra de sangre entera venosa, extraída con un sistema de extracción de sangre que contenga EDTA como anticoagulante.
- La muestra de sangre debe transportarse en un contenedor oscuro, protegido de la luz y no expuesto a temperaturas elevadas. Un contenedor de transporte adecuado es, por ejemplo, una caja de poliestireno extruido con material refrigerante. Las mejores condiciones de transporte serían inferiores a 25 °C si el transporte no durará más de 6 horas después de la donación.
- Para el transporte y el tiempo de almacenamiento de más de 6 horas, la sangre debe mantenerse a 2 - 8 °C.
- No se puede congelar y descongelar la muestra de sangre.
- En condiciones óptimas la muestra de sangre debería ser reciente, es decir, no deberían haber pasado más de seis horas entre la extracción y el análisis de la muestra.
- Las muestras de sangre se pueden utilizar hasta 24 horas después de la extracción si se almacenan en el frigorífico a 2 - 8 °C.
- Los tubos con las muestras de sangre se deben voltear con suavidad de 8 a 10 veces antes de ser utilizados.
- No utilice muestras de sangre coagulada.

11 Procedimiento de análisis

AVISO *El pipeteado inverso es fundamental para la precisión, en particular cuando se dispensan volúmenes de muestra viscosos y muy pequeños. Para el pipeteado de sangre completa, recomendamos emplear una pipeta electrónica calibrada que esté preprogramada para funcionar en modo de pipeteado inverso.*
Si no hay disponible una pipeta electrónica, siga las presentes instrucciones para llevar a cabo un pipeteado inverso manual:
- *Presione el botón de funcionamiento hasta el segundo tope. Deje que el botón de funcionamiento ascienda por completo; la muestra sobrante se aspirará a la punta.*
- *Presione el botón de funcionamiento hasta el primer tope para expulsar un volumen concreto de sangre, dejando así la muestra sobrante dentro de la punta.*
- *Elimine la punta de la pipeta que contiene la muestra de sangre sobrante en un contenedor para residuos médicos.*

Tenga en cuenta que no debe utilizar el pipeteado inverso para soluciones de anticuerpos ni soluciones tampón.

11.1 Tinción
- Voltee con suavidad de 8 a 10 veces el tubo de extracción de sangre con EDTA que contiene la muestra de sangre.
- Pase con la pipeta 20 µl de la muestra de sangre entera con EDTA al fondo de un tubo de muestra y evite manchar la pared interior del tubo con la sangre de la punta de la pipeta. Deseche la punta de la pipeta.
- Añada 10 µl de mAb CD4-PE directamente en la muestra de sangre y mézclelo suavemente, evitando manchar la pared interior del tubo con la sangre de la punta de la pipeta. Deseche la punta de la pipeta.
- Añada 10 µl de mAb CD45-PE-Cy5 directamente en la muestra de sangre y mézclelo suavemente, evitando manchar la pared interior del tubo con la sangre de la punta de la pipeta. Deseche la punta de la pipeta.
- Incube la mezcla durante 15 minutos a 15 - 30 °C en la oscuridad.
- Añada 400 µl de la solución amortiguadora 1 y agite brevemente con la agitadora vorticial, o bien golpee suavemente el tubo para mezclar la muestra. Deseche la punta de la pipeta.
- Añada 400 µl de la solución amortiguadora 2 y agite brevemente con la agitadora vorticial, o bien golpee suavemente el tubo para mezclar la muestra. Deseche la punta de la pipeta.
- Agite brevemente mediante agitadora vorticial, o bien golpee suavemente el tubo antes del análisis con el CyFlow™ Counter.

AVISO *Tras añadir la solución amortiguadora 1, las muestras pueden almacenarse hasta 2 horas a 2 - 8 °C a oscuras. No utilice la muestra asado este periodo o si no se ha preparado correctamente. Las muestras se deben analizar dentro de un periodo máximo de 10 minutos tras la adición de la solución amortiguadora 2.*

11.2 Análisis de la muestra
En las instrucciones de uso de CyFlow™ Counter (CY-S-3022IFUEN) puede consultar el modo del empleo del equipo antes de analizar la muestra. Los procedimientos de puesta en marcha y el proceso de control de calidad interno deben completarse con éxito antes de iniciar el análisis de la muestra.
- Elija y cargue la configuración para la medición de CD4% desde la barra de menú de CyFlow™ Counter.
- Inserte el tubo de muestras con la muestra de sangre preparada en el acoplamiento para muestras.
- Inicie la medición.
- Tras el análisis de la muestra, el dispositivo se detiene y se limpia automáticamente.
- Retire y deseche la muestra de sangre conforme a los procedimientos locales de seguridad en laboratorio frente a riesgos biológicos.

No inicie una segunda medición con el mismo tubo de muestras. Si se requiere una repetición de la medición, se debe preparar un segundo tubo de muestras conforme a 11.1.

11.3 Adquisición y análisis de datos
El análisis de datos con CyFlow™ Counter solo es posible si el control de calidad interno del instrumento ha resultado satisfactorio. Consulte las instrucciones de uso del CyFlow™ Counter (CY-S-3022IFUEN) para realizar, si fuera necesario, el procedimiento de inicio y mantenimiento del dispositivo. Los resultados de contar el número de células CD4-T, el porcentaje de células CD4 y el recuento de linfocitos se presentarán en la pantalla. Si las agrupaciones de células teñidas no se pueden diferenciar claramente de las señales de ruido de fondo en el diagrama de dispersión lateral de CD45 (véase la figura 2), o bien no se pueden diferenciar claramente las agrupaciones de células positivas para CD4, las células negativas para CD4 y los monocitos en el diagrama de dispersión lateral de CD4, entonces habrá que repetir la preparación de la muestra y la medición.

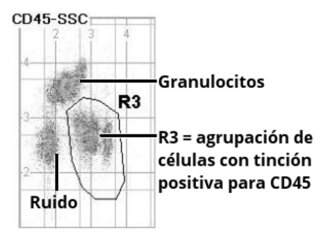


Figura 1: Ejemplo de agrupaciones bien diferenciadas de granulocitos, otros leucocitos con tinción positiva para CD45 y señales de ruido.

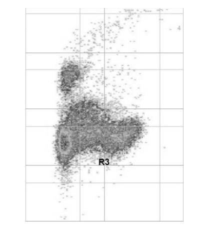


Figura 2: Ejemplo de mala separación entre ruido de fondo y los leucocitos teñidos con CD45 positivo. Esta muestra no es analizable.

Los resultados serán incorrectos si el posicionamiento de la puerta de las células teñidas con CD45 positivo (R3) no se realiza con precisión.

12 Cálculo de los resultados del análisis
El cálculo de la concentración celular forma parte del análisis de datos informático, el cual suministra el resultado en CD4%, células CD4+ por µl de muestra de sangre y linfocitos por µl de muestra de sangre después de cada medición.
Para más información, consulte las instrucciones de uso de CyFlow™ Counter (CY-S-3022IFUEN).

13 Interpretación de los resultados
El virus de la inmunodeficiencia humana (VIH) es uno de los principales motivos del agotamiento de las células CD4, que debilita el sistema inmunitario. Los resultados deben ser interpretados por los médicos, en conjunción con las pautas de tratamiento del VIH aplicables localmente.

14 Procedimiento de control
Se debe realizar un control de calidad apropiado conforme a las normativas locales y nacionales. Se han comercializado diversas muestras de sangre estabilizada, pero solo unas pocas son adecuadas cuando se utilizan determinados citómetros de flujo. Si no se cumple el criterio de aceptación del material de control específico, no prosiga con el análisis de la muestra del paciente y póngase en contacto con su representante local de Sysmex.
Para más información sobre el uso de material de control adecuado, póngase en contacto con su representante local de Sysmex.

15 Eficacia diagnóstica
15.1 Especificidad
El anticuerpo MEM-241 reconoce el antígeno CD4, una glucoproteína transmembranaria de 55 kDa expresada en un subconjunto de linfocitos T (células T cooperadoras) y también en monocitos, macrófagos y granulocitos. Congreso de HCDM (anteriormente HLDA VIII), mayo de 2006, Quebec, Canadá. Código WS: M241.
El anticuerpo MEM-28 reacciona con todas las formas alternativas de antígeno humano CD45 (antígeno leucocitario común), una proteína transmembranaria de tipo I monocatenaria de 180-220 kDa con elevada concentración en todas las células de origen hematopoyético, excepto los eritrocitos y las plaquetas. HLDA III. Código WS: NL 833a.

15.2 Capacidad de detección
La evaluación del límite de inclusión (LI), el límite de detección (LD) y el límite de cuantificación (LC) se realizó de acuerdo con las indicaciones de la directriz CLSI EP17-A2.
LI: ≤50% del LD
LD: ≤20 células CD4/µl
LC: ≤40 células CD4/µl

15.3 Repetibilidad
Variación intra e interanalítica:
Los valores de coeficiente de variación (CV) suponen menos del 10% para valores de CD4 ≥ 200 células T CD4/µl.
Los valores de coeficiente de variación (CV) suponen menos al 10% para valores de CD4% ≥ 25%.
Los valores de coeficiente de variación (CV) suponen menos del 15% para valores absolutos de CD4 inferiores a 200 células T CD4/µl.
Los valores de desviación estándar (SD) ≤ 2,5% para valores de CD4% inferiores al 25%.

15.4 Linealidad
El intervalo de medición del ensayo y la linealidad de la medición son válidos en el intervalo:
CD4 absoluto: 40-2500 células T CD4/µl
CD4%: 4 – 60%

15.5 Concordancia
Las muestras de sangre entera con EDTA se tiñeron con el kit CD4% easy count y se analizaron en el CyFlow™ Counter (CyView™ 2.11). Se compararon los valores de CD4 absoluto y CD4% con los resultados de un BD FACSCalibur utilizando BD Tritest CD3/CD4/CD45 y tubos BD Trucount y se puso de manifiesto una divergencia del -22,56% (CD4 absoluto) y del +2,1% (CD4%) entre ambos métodos.

16 Limitaciones

CUIDADO ⚠ *El uso de los kits de Sysmex Partec CD4% easy count (05-8405) no está validado para pacientes pediátricos y adolescentes.*

CUIDADO ⚠ *Las mediciones de los pacientes de CD4 y CD4% realizadas con los ensayos de Sysmex Partec no deben utilizarse junto con las medidas obtenidas de otros métodos del fabricante para determinar CD4 y CD4%. Use los ensayos solo con los instrumentos de Sysmex Partec. Los valores obtenidos usando otros equipos o ensayos no son intercambiables.*

Se analizaron diferentes sustancias endógenas y exógenas conforme a la directriz CLSI EP07-A2 para detectar posibles efectos de interferencia que pudieran influir en el análisis de la muestra de sangre. Finalmente, se observó un ≥18% de hemólisis (provocada por el uso del protocolo recomendado en CLSI EP07-A2, apéndice G, G1, «Procedimiento de choque osmótico») como muestra de un efecto de interferencia. Por ese motivo, se deben rechazar las muestras hemolizadas. Para la determinación de CD4 y CD4% en muestras de sangre total con los ensayos CyFlow™ Counter, no se debe utilizar ningún otro anticoagulante que el EDTA.
Si tiene alguna pregunta más con respecto al funcionamiento del producto, póngase en contacto con su representante local de Sysmex.

17 Referencias bibliográficas
1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondière, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
2) L. Zijenah, G. Kadzirange, S. Madzime, M. Borok, C. Mudiwa, O. Tobaiwa, M. Mucheche, S. Rusakaniko, D. Katzenstein. (2006). Affordable flow cytometry for enumeration of absolute CD4+ T-lymphocytes to identify subtype C HIV-I infected adults requiring antiretroviral therapy (ART) and monitoring response to ART in a resource-limited setting. Journal of Translational Medicine 4:33
3) H. Karcher, D. Bohning, R. Downing, S. Mashate, G. Harms. (2006). Comparison of two alternative methods for CD4+ T-cell determination (Coulter manual CD4 count and CyFlow) against standard dual platform flow cytometry in Uganda. Clinical Cytometry, 70B (3):163-69
4) S. Kohreanudom, S. Dettraira. (2012). Evaluation of the Partec Single-Platform Volumetric CyFlow® Counter System for Determining Percentage and Absolute Numbers of CD4 T Lymphocytes in HIV/AIDS Thai Patients. Thai AIDS J, 25:1-10
5) D. Wade, P.A. Diaw, G. Daneau, M. Camara, T.N. Dieye, S. Mboup, L. Kestens. (2013). CD4 T-Cell Enumeration in a Field Setting: Evaluation of CyFlow Counter Using the CD4 Easy Count Kit-Dry and Pima CD4 Systems. PLoS ONE, 8(9): e75484

18 Contacto del fabricante
Fabricante
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Alemanha
www.sysmex-partec.com
Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Número de versión de las instrucciones de uso y fecha de publicación
Revisión: Rev-07_10-09-2020_CN 1563
Publicada por: Sysmex Partec GmbH

Puede consultar la ficha técnica de seguridad de este producto en www.sysmex-partec.com/services.

Instruções de utilização

Identificação do reagente IVD
Nome do produto:
CD4% easy count kit   REF 05-8405

Conteúdo:
Recipiente contendo CD4 mAb PE   REF 05-8405-01
Recipiente contendo CD45 mAb PE-Cy5   REF 05-8405-02
Frasco contendo Tampão 1   REF 05-8405-03
Frasco contendo Tampão 2   REF 05-8405-04

1 Especificação

Especificidade	CD4 humano	CD45 humano
Isótipo	IgG1	IgG1
Conteúdo	100 testes	100 testes
Fluorocromo	PE	PE-Cy5
λ excitação (nm)	532 / 488	532 / 488
Máximo de emissão (nm)	578	670

2 Uso previsto
IVD Para uso no diagnóstico in vitro.
O CD4% easy count kit é um teste IVD manual quantitativo com quatro componentes, para a marcação de leucócitos e de uma subpopulação de linfócitos em sangue total EDTA venoso de adulto, que podem ser posteriormente enumerados no citómetro de fluxo IVD Sysmex Partec CyFlow™ Counter.
A concentração de células CD4 T e CD4% de linfócitos em amostras de sangue são indicadores útil para o iníciação ou o acompanhamento do tratamento para pacientes vom VIH positivo em conjugação com outros resultados laboratoriais e clínicos.
O teste destina-se a ser realizado por profissionais de saúde devidamente qualificados.

3 Princípio do método de análise
Uma alíquota de uma amostra de sangue total EDTA é misturada com dois anticorpos (CD4 e CD45), cada um deles conjugado com um fluorocromo diferente para marcar populações de células específicas. Após um tempo de incubação fixo, as duas soluções tampão são adicionadas e a amostra fica pronta para análise no citómetro de fluxo CyFlow™ Counter.
A fonte de luz excita o corante fluorescente ligado à célula corada e a luz emitida é detectada enquanto um volume preciso de amostra de sangue passa pelo instrumento. A concentração das populações de células específicas é calculada pelo software integrado.
Para mais informações, consulte as instruções de utilização do CyFlow™ Counter (CY-S-3022IFUEN).

4 Armazenamento e prazo de validade após a primeira abertura
1. Armazenamento:
Armazene os reagentes tampão e de anticorpos entre 2 - 8 °C no escuro. Não congele nem exponha os reagentes a temperaturas elevadas e mantenha-os protegidos da luz solar direta.
2. Prazo de validade após a primeira abertura:
Feche sempre o frasco após a utilização e utilize pontas de pipeta novas de cada vez que o reagente é amostrado, para evitar a contaminação.
Sob estas condições de armazenamento, o CD4% easy count kit irá permanecer estável até à data de validade impressa no rótulo do kit.

5 Componentes
O CD4 mAb PE e o CD45 mAb PE-Cy5 são anticorpos monoclonais murinos fornecidos em tampão PBS com 0,2% de BSA e 0,09% de azida de sódio. Tampão 1 e tampão 2 são soluções baseadas em PBS contendo 0,09% de azida de sódio.

6 Indício de deterioração
As soluções tampão e de anticorpos são líquidos transparentes. Não utilize os reagentes caso surja qualquer tipo de turbidez ou contaminação.
Para questões relativas ao desempenho ou qualidade do produto, entre em contacto com o representante local da Sysmex para obter suporte técnico.

7 Precauções e advertências
Os reagentes contêm 14 mM de azida de sódio como conservante. A baixa concentração de azida de sódio não exige a rotulagem como substância perigosa, mas é necessário respeitar as precauções de segurança normais relativas ao manuseamento dos produtos químicos. Consulte a ficha de segurança para obter uma lista completa das advertências e precauções.

8 Additional required equipment

Instrumento necessário:	CyFlow™ Counter (REF No.: CY-S-3022)
Equipamento necessário:	Tubos de amostra de 3.5 ml (REF No.: 04-2000) Uma pipeta verificada de 10 µl e pontas de pipeta Uma pipeta verificada de 20 µl e pontas de pipeta Uma pipeta verificada de 100 - 1000 µl e pontas de pipeta Sistema de colheita de sangue venoso com EDTA como anticoagulante Cronómetro

9 Preparação do reagente
CD4 mAb PE (REF No.: 05-8405-01), O reagente de anticorpos está pronto a usar.
CD45 mAb PE-Cy5 (REF No.: 05-8405-02), O reagente de anticorpos está pronto a usar.
Tampão 1 (REF No.: 05-8405-03), A solução tampão está pronta a usar.
Tampão 2 (REF No.: 05-8405-04), A solução tampão está pronta a usar.

10 Colheita, manuseamento e armazenamento da amostra principal

ADVERTÊNCIA ⚠ *Todas as amostras biológicas e materiais são considerados como perigosos e devem ser manuseados como sendo capazes de transmitir infeções. É necessário aplicar precauções de segurança e procedimentos de manuseamento adequados em conformidade com as leis e regulamentos aplicáveis.*

- A amostra de sangue tem de ser uma amostra de sangue total venoso, recolhida com um sistema de colheita de sangue, que contém EDTA como anticoagulante.
- A amostra de sangue tem de ser transportada num recipiente escuro, protegida da luz e não exposta a temperaturas elevadas. Um recipiente de transporte adequado é, p. ex., uma caixa de espuma de poliestireno com material de refrigeração. As melhores condições de transporte seriam inferiores a 25 °C se o transporte não durar mais de 6 horas após a doação.
- Para transporte e tempo de armazenamento superior a 6 horas, o sangue deve ser mantido a 2 - 8 °C.
- Não congele e descongele a amostra de sangue.
- A fim de assegurar as melhores condições, a amostra de sangue deve ser fresca, ou seja, o tempo de colheita e análise da mesma não pode ser superior a seis horas.
- As amostras de sangue podem ser utilizadas até 24 horas após a colheita, desde que tenham sido armazenadas no frigorífico entre 2 - 8 °C.
- Antes da utilização, os tubos de amostra que contêm o sangue devem ser invertidos cuidadosamente 8 - 10 vezes.
- Não utilize amostras de sangue coagulado

11 Procedimento de análise

NOTA *A pipetagem reversa é fundamental para a precisão, sobretudo ao dispensar volumes de amostra viscosos e muito reduzidos. Para a pipetagem de sangue total, recomendamos a utilização de uma pipeta eletrónica calibrada pré-programada para operar em modo de pipetagem reversa.*
Se não estiver disponível uma pipeta eletrónica, seguir estas instruções para a pipetagem reversa manual:
- *Premir o botão de operação até ao segundo ponto de retenção. Deixar o botão de operação subir completamente; o excesso da amostra é aspirado para a ponta.*
- *Premir o botão de operação até ao primeiro ponto de retenção para expulsar um volume exato de sangue, deixando o excesso da amostra na ponta.*
- *Deitar a ponta da pipeta com a amostra de sangue em excesso no contentor de lixo hospitalar.*

Ter em atenção que a pipetagem reversa não pode ser usada para anticorpos ou soluções-tampão.

11.1 Coloração
- Inverta cuidadosamente a amostra de sangue no tubo de colheita de sangue EDTA 8 - 10 vezes.
- Pipete 20 µl de amostra de sangue total EDTA até ao fundo de um tubo de amostra, evitando deixar um rasto de sangue na parede interior do tubo com a ponta de pipeta. Descarte a ponta da pipeta.
- Adicione 10 µl de CD4 mAb PE diretamente na amostra de sangue e misture cuidadosamente, evitando deixar um rasto de sangue na parede interior do tubo com a ponta de pipeta. Descarte a ponta da pipeta.
- Adicione 10 µl de CD45 mAb PE-Cy5 diretamente na amostra de sangue e misture cuidadosamente, evitando deixar um rasto de sangue na parede interior do tubo com a ponta de pipeta. Descarte a ponta da pipeta.
- Incube a mistura durante 15 minutos noescuro à 15 - 30 °C.
- Adicione 400 µl de tampão 1 e centrifugue brevemente ou bata cuidadosamente no tubo para misturar a amostra. Descarte a ponta da pipeta.
- Adicione 400 µl de tampão 2 e centrifugue brevemente ou bata cuidadosamente no tubo para misturar a amostra. Descarte a ponta da pipeta.
- Centrifugue brevemente ou bata cuidadosamente no tubo antes de efetuar a análise com o CyFlow™ Counter.

NOTA *Após a adição do tampão 1, as amostras podem ser armazenadas num ambiente escuro e a 2 - 8 °C durante um período máximo de 2 horas. Não use a amostra depois desse período ou caso esta não tenha sido devidamente preparada.*
Após a adição do tampão 2, as amostras têm de ser analisadas dentro de 10 minutos.

11.2 Análise da amostra
Consulte as instruções de utilização do CyFlow™ Counter para obter informações sobre como operar o instrumento antes de analisar a amostra. Os procedimentos de arranque e o procedimento interno de controlo da qualidade têm de ser concluídos com êxito antes da análise da amostra.
- Escolha e carregue a configuração para medição de CD4% na barra de menu do CyFlow™ Counter.
- Insira o tubo da amostra com a amostra de sangue preparada na porta de amostragem.
- Inicie a medição.
- Após o processamento da amostra, o instrumento para e limpa-se automaticamente.
- Retire e elimine a amostra de sangue de acordo com os procedimentos de segurança locais do laboratório em termos de perigo biológico.

Não inicie uma segunda medição com o mesmo tubo de amostra. Se for necessária uma duplicação ou repetição da medição, terá de ser preparado um segundo tubo de amostra tal como descrito em 11.1.

11.3 Aquisição e análise de dados
A análise de dados com o CyFlow™ Counter só é possível caso o controlo de qualidade interno do instrumento tenha sido executado com êxito.
Consulte as instruções de utilização do CyFlow™ Counter (CY-S-3022IFUEN) para informações sobre o procedimento de arranque e a manutenção do dispositivo, se necessário.
Os resultados da contagem do número de células CD4-T, determinando a porcentagem de células CD4 e a contagem de linfócitos serão apresentados na tela. Se os aglomerados das células coradas contra os sinais de ruído de fundo no gráfico de pontos CD45 – SSC não forem claramente separáveis (ver figura 2), ou se os aglomerados de células positivas CD4, células negativas CD4 e monócitos no gráfico de pontos CD4 – SSC não forem claramente separáveis, a preparação e medição da amostra têm de ser repetidas..

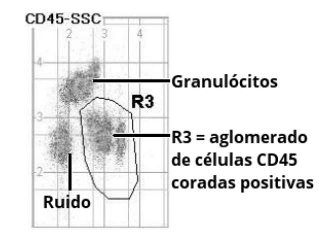


Figura 1: Exemplo de aglomerados de granulócitos bem separados, outros leucócitos corados positivos CD45 e sinais de ruído.

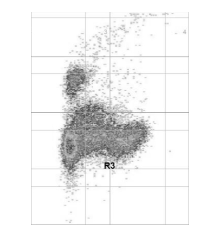


Figura 2: Exemplo de má separação entre ruído e leucócitos corados positivos CD45. Esta amostra não é analisável.

Os resultados serão incorretos se o posicionamento da porta de células CD45 positivas (R3) não for feito com precisão.

12 Cálculo dos resultados da análise
O cálculo da concentração de células faz parte da análise de dados baseada em software, que fornece o resultado em células CD4% e CD4+ por µl de amostra de sangue e linfócitos por µl de amostra de sangue, após cada medição.
Para mais informações, consulte as instruções de utilização do CyFlow™ Counter (CY-S-3022IFUEN).

13 Interpretação dos resultados
O vírus da imunodeficiência humana (VIH) é uma das principais razões para a depleção de células CD4, que debilita o sistema imunológico. Os resultados devem ser interpretados pelos médicos, em conjunto com as diretrizes de tratamento do VIH aplicáveis localmente.

14 Procedimento de controlo
Deve ser realizado um controlo da qualidade apropriado de acordo com regulamentos locais e nacionais. Encontra-se disponível no mercado uma variedade de amostras de sangue estabilizado, mas poucas são adequadas para a utilização de certos citómetros de fluxo.
Se não for cumprido o critério de aceitação do material de controlo individual, não avance para o teste da amostra do paciente e entre em contacto com o seu representante local da Sysmex.
Para mais informações acerca do uso de material de controlo adequado, entre em contacto com o seu representante local da Sysmex.

15 Características de desempenho
15.1 Especificidade
O anticorpo MEM-241 reconhece o antigénio CD4, uma glicoproteína transmembrana de 55 kDa expressa num subconjunto de linfócitos T (células T „auxiliares") e também em monócitos, granulócitos e macrófagos do tecido. Conferência sobre HCDM (o anterior HLDA VIII), maio de 2006, Quebeque, Canadá; código WS M241
O anticorpo MEM-28 reage com todas as formas alternativas de antigénio CD45 humano (antigénio leucocitário comum), uma proteína transmembrana tipo I de cadeia simples de 180-220 kDa, expressa a nível superior em todas as células de origem hematopoiética, exceto nos eritrócitos e plaquetas. HLDA III; código WS NL 833a

15.2 Capacidade de deteção
A avaliação para Limite de valores em branco (LoB), Limite de deteção (LoD) e Limite de quantificação (LoQ) foi realizada de acordo com as especificações da diretriz CLSI EP17-A2.
LoB: ≤50% of LoD
LoD: ≤20 CD4 células/µl
LoQ: ≤40 CD4 células/µl

15.3 Repetibilidade
Variação intra e entre ensaios:
Os valores CV são inferiores a 10% para valores CD4 de 200 células T CD4/µl ou superiores.
Os valores CV são inferiores a 10% para valores CD4% de 25% ou superiores.
Os valores CV são inferiores a 15% para valores absolutos de CD4 inferiores a 200 células T CD4/µl.
Os valores do desvio padrão (SD) ≤ 2,5% para valores de CD4% são inferiores a 25%.

15.4 Linearidade
A gama de medição do ensaio e linearidade da medição são válidas ao longo da gama:
CD4 absoluto:: 40-2500 CD4 T-células/µl
CD4%: 4 – 60%

15.5 Veracidade
Uma amostra de sangue total EDTA foi corada com o CD4% easy count kit e analisada no CyFlow™ Counter (CyView™ 2.11). Os valores de CD4 absoluto e CD4% foram comparados com os resultados de um BD FACSCalibur usando BD Tritest CD3/CD4/CD45 e tubos BD Trucount, tendo-se revelado um desvio de -22,56% (CD4 absoluto) e +2,1% (CD4%) entre ambos os métodos.

16 Limitações

CUIDADO ⚠ *Os kits Sysmex Partec CD4% easy count (05-8405) não foram validados para utilização com pacientes pediátricos e adolescentes.*

CUIDADO ⚠ *As medições de paciente de CD4 e de CD4% feitas com os ensaios Sysmex Partec não devem ser usadas de forma independente com outros métodos do fabricante para determinar CD4 e CD4%.*
Use os ensaios apenas com os instrumentos Sysmex Partec. Os valores obtidos usando outros equipamentos ou ensaios podem não ser intercambiáveis.

Foram testadas diversas substâncias endógenas e exógenas relativamente à diretriz CLSI EP07-A2 para identificar possíveis efeitos de interferência que possam exercer influência sobre a análise da amostra de sangue.
Finalmente, observou-se ≥ 18% de hemólise (induzida usando o protocolo recomendado na CLSI EP07-A2, Apêndice G, G1 - Procedimento de Choque Osmótico) para demonstrar um efeito de interferência. Por esse motivo, deverão ser rejeitadas amostras hemolisadas. Para a determinação de CD4 e CD4% em amostras de sangue total com ensaios CyFlow™ Counter, nenhum outro anticoagulante que o EDTA deve ser usado. Para outras questões relativas ao desempenho do produto, entre em contacto com o seu representante local da Sysmex.

17 Referências bibliográficas
1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondière, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
2) L. Zijenah, G. Kadzirange, S. Madzime, M. Borok, C. Mudiwa, O. Tobaiwa, M. Mucheche, S. Rusakaniko, D. Katzenstein. (2006). Affordable flow cytometry for enumeration of absolute CD4+ T-lymphocytes to identify subtype C HIV-I infected adults requiring antiretroviral therapy (ART) and monitoring response to ART in a resource-limited setting. Journal of Translational Medicine 4:33
3) H. Karcher, D. Bohning, R. Downing, S. Mashate, G. Harms. (2006). Comparison of two alternative methods for CD4+ T-cell determination (Coulter manual CD4 count and CyFlow) against standard dual platform flow cytometry in Uganda. Clinical Cytometry, 70B (3):163-69
4) S. Kohreanudom, S. Dettraira. (2012). Evaluation of the Partec Single-Platform Volumetric CyFlow® Counter System for Determining Percentage and Absolute Numbers of CD4 T Lymphocytes in HIV/AIDS Thai Patients. Thai AIDS J, 25:1-10
5) D. Wade, P.A. Diaw, G. Daneau, M. Camara, T.N. Dieye, S. Mboup, L. Kestens. (2013). CD4 T-Cell Enumeration in a Field Setting: Evaluation of CyFlow Counter Using the CD4 Easy Count Kit-Dry and Pima CD4 Systems. PLoS ONE, 8(9): e75484

18 Contacto do fabricante
Fabricante
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Alemanha
www.sysmex-partec.com
Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Número de versão das instruções de utilização e data da pu blicação
Revisão: Rev-07_10-09-2020_CN 1563
Publicado por: Sysmex Partec GmbH

A ficha de segurança deste produto está disponível em www.sysmex-partec.com/services.

Инструкция по эксплуатации

Идентификация реагента для диагностики in vitro (IVD)
Наименование продукта
CD4% easy count kit   REF 05-8405

Содержимое:
Ампула, содержащая CD4 mAb PE   REF 05-8405-01
Ампула, содержащая CD45 mAb PE-Cy5   REF 05-8405-02
Флакон, содержащий буфер 1   REF 05-8405-03
Флакон, содержащий буфер 2   REF 05-8405-04

1 Спецификация

Специфичность	CD4 человека	CD45 человека
Изотип	IgG1	IgG1
Содержимое	100 тестов	100 тестов
Флуорохром	PE	PE-Cy5
λ возбуждение (нм)	532 / 488	532 / 488
Максимальная эмиссия (нм)	578	670

2 Предполагаемое использование
IVD Для использования в диагностике in vitro.
Набор CD4% easy count kit — это четырехкомпонентный набор, предназначенный для ручного количественного анализа in vitro, используемый для мечения лейкоцитов и субпопуляций лимфоцитов в цельной венозной крови с ЭДТА взрослых пациентов и их последующего подсчета с помощью проточного цитометра для диагностики in vitro CyFlow™ Counter компании Sysmex Partec. Концентрация CD4 Т-клеток и CD4% лимфоцитов в образцах крови являются полезными показателями для начала или последующего лечения пациентов с ВИЧ-инфекцией в сочетании с другими лабораторными и клиническими данными.
Анализ должен проводиться обученными специалистами здравоохранения.

3 Принцип метода подсчета
Аликвоту образца цельной крови с ЭДТА смешивают с двумя типами антител (CD4 и CD45), конъюгированных с разными флуорохромами для мечения клеток определенных популяций. После инкубирования в течение фиксированного времени добавляют два буферных раствора, после чего образец готов для анализа на проточном цитометре CyFlow™ Counter.
Источник света возбуждает флуоресцентный краситель, связанный с окрашенной клеткой, и излучаемый свет обнаруживается, когда точный объем образца крови проходит через инструмент. Концентрация клеток определенных популяций рассчитывается с помощью интегрированного программного обеспечения.
Для получения дополнительной информации см. ИЭ цитометра CyFlow™ Counter (CY-S-3022IFUEN).

4 Хранение и срок годности после первого вскрытия
1. Хранение:
Антитела и буферные реагенты следует хранить при температуре 2 - 8 °C в темном месте. Реагенты нельзя замораживать, подвергать воздействию высоких температур и прямых солнечных лучей.
2. Срок годности после первого вскрытия:
Следует всегда закрывать флакон после использования и каждый раз при взятии реагента пользоваться новым наконечником для пипетки с целью предотвращения контаминации.
При таких условиях хранения реагенты набора CD4% easy count kit будут стабильны до окончания срока годности, указанного на его этикетке.

5 Компоненты
CD4 mAb PE и CD45 mAb PE-Cy5 представляют собой моноклональные мышиные антитела в фосфатно-солевом буфере (PBS), содержащем 0,2 % БСА и 0,09 % азида натрия. Буфер 1 и буфер 2 представляют собой растворы на основе PBS, содержащие 0,09 % азида натрия.

6 Признаки ухудшения качества
Антитела и буферные растворы представляют собой прозрачную жидкость. Не следует использовать реагенты при появлении любого рода помутнения или признаков контаминации.
При наличии вопросов о характеристиках или качестве продукта обращайтесь в службу технической поддержки местного представительства компании Sysmex.

7 Предупреждения и меры предосторожности
Реагенты содержат 14 мМ азида натрия в качестве консерванта. Низкая концентрация азид натрия не требует маркировки опасности, однако при их использовании следует соблюдать стандартные меры предосторожности по безопасному обращению с химическими веществами. Полный перечень предупреждений и мер предосторожности приведен в паспорте безопасности (ПБ) продукта.

8 Дополнительное требуемое оборудование

Требуемый прибор:	CyFlow™ Counter (REF No.: CY-S-3022)
Требуемое оборудование:	пробирки объемом 3,5 мл (REF No.: 04-2000) поверенная пипетка объемом 10 мкл и наконечники для пипетки поверенная пипетка объемом 20 мкл и наконечники для пипетки поверенная пипетка объемом 100 - 1000 мкл и наконечники для пипетки система для сбора венозной крови с ЭДТА в качестве антикоагулянта секундомер

9 Приготовление реагента
CD4 mAb PE (REF No.: 05-8405-01), Реагент с антителами готов к использованию.
CD45 mAb PE-Cy5 (REF No.: 05-8405-02), Реагент с антителами готов к использованию.
буфер 1 (REF No.: 05-8405-03), Буферный раствор готов к использованию.
буфер 2 (REF No.: 05-8405-04), Буферный раствор готов к использованию.

10 Взятие основного образца, обращение с ним и его хранение

ПРЕДУПРЕЖДЕНИЕ ⚠ *Все биологические образцы и материалы считаются биологически опасными, с ними следует обращаться как с потенциальным источником инфекции. При обращении с ними следует соблюдать надлежащие меры предосторожности и требования безопасности в соответствии с применимыми законами и нормативами*

- Образцом должен служить образец цельной венозной крови, взятый с помощью системы для сбора крови, содержащей ЭДТА в качестве антикоагулянта.
- Образец крови следует транспортировать в непрозрачном контейнере, защищать от воздействия света и не подвергать воздействию высоких температур. Подходящим транспортным контейнером является, например, пенопластовый (Styrofoam) контейнер с охлаждающим материалом. Лучшие транспортные условия будут ниже 25 °C, если транспорт не займет больше 6 часов после пожертвования.
- Для транспортировки и хранения более 6 часов кровь должна храниться при температуре 2 - 8 °C.
- Нельзя замораживать и размораживать образец крови.
- Для получения наилучших результатов образец крови должен быть свежим, т. е. между его взятием и анализом должно пройти не более шести часов.
- Образцы крови можно использовать в течение 24 часов после взятия, если они хранятся в холодильнике при температуре 2 - 8 °C.
- Перед началом анализа пробирку с образцом крови следует аккуратно перевернуть от 8 - 10 раз.
- Использование тромбов запрещено.

11 Процедура анализа

ПРИМЕЧАНИЕ *Обратное пипетирование крайне важно для обеспечения точности, особенно при дозировании проб очень малого объёма. Рекомендуется использовать калиброванную электронную пипетку, запрограммированную на работу в режиме обратного пипетирования.*
Если электронная пипетка недоступна, выполните обратное пипетирование вручную следующим образом:
- *нажмите на кнопку управления до второго упора. Дайте кнопке управления полностью подняться, при этом избыток пробы втягивается в кончик.*
- *нажмите на кнопку управления до первого упора, чтобы выдавить точный объём крови, оставив избыток пробы в кончике.*
- *Выбросьте кончик пипетки с избытком пробы крови в контейнер для медицинских отходов.*

Обратите внимание, что обратное пипетирование нельзя использовать для растворов антител и буферных растворов.

11.1 Окрашивание
Переверните пробирку с ЭДТА и образцом крови примерно 8 - 10 раз.
Возьмите пипеткой 20 мкл образца цельной крови с ЭДТА со дна пробирки, не оставляя наконечником пипетки потеков крови на стенке пробирки. Отбросьте наконечник пипетки.
Добавьте 10 мкл раствора антитела CD4 mAb PE непосредственно в образец крови и аккуратноперемешайте, избегая образования потеков крови на стенке пробирки. Отбросьте наконечник пипетки.
Добавьте 10 мкл раствора антитела CD45 mAb PE-Cy5 непосредственно в образец крови и аккуратно перемешайте, избегая образования потеков крови на стенке пробирки. Отбросьте наконечник пипетки.
Инкубируйте смесь в течение 15 минут при температуре от 15 - 30 °C в темноте.
Добавьте 400 мкл буфера 1 и встряхните образец или аккуратно перемешайте вихревыми движениями. Отбросьте наконечник пипетки.
Добавьте 400 мкл буфера 2 и встряхните образец или аккуратно перемешайте вихревыми движениями. Отбросьте наконечник пипетки.
Непосредственно перед анализом с помощью цитометра CyFlow™ Counter образец следует встряхнуть или аккуратно перемешать вихревыми движениями.

ПРИМЕЧАНИЕ *После добавления буфера 1 образцы можно хранить не более 2 часов при температуре 2 - 8 °C в темном месте. Не используйте образцы, хранившиеся более длительное время или неправильно приготовленные. После добавления буфера 2 образцы должны быть проанализированы в течение 10 минут.*

11.2 Анализ образца
Пожалуйста, обратитесь к IFU CyFlow™ Counter о том, как работать с прибором Перед анализом образцов. Процедуры запуска и внутренняя процедура контроля качества должны быть успешно завершено перед анализом образца.
- Выберите и загрузите конфигурацию для измерения CD4% в строке меню CyFlow™ Counter.
- Вставьте пробирку с приготовленным образцом крови в соответствующий держатель.
- Начните анализ.
- После анализа образца прибор прекращает работу и очищается автоматически.
- Извлеките и утилизируйте образец крови в соответствии с местными правилами по биологической безопасности при работе в лабораториях.

Не проводите повторный анализ с использованием той же самой пробирки с образцом. Если нужно выполнить дублирующее или повторное измерение, вторую пробирку для анализа подготовьте согласно п 11.1.

11.3 Сбор и анализ данных
Анализ данных с помощью цитометра CyFlow™ Counter возможен только при успешном выполнении процедуры внутреннего контроля качества прибора.
Информация, необходимая для запуска и технического обслуживания прибора, содержится в ИЭ цитометра CyFlow™ Counter (CY-S-3022IFUEN).
На экране появится результат подсчета CD4 т-клеток, процент CD4 и общее количество лимфоцитов.

Рисунок 1: Пример хорошего отделения кластеров гранулоцитов и других положительно окрашенных CD45-лейкоцитов от шумовых сигналов.

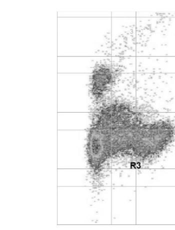


Рисунок 2: Пример плохого разделения мусора и положительных окрашенных лейкоцитов CD45. Этот образец не анализируемых.

Результаты будут неверными, если позиционирование ворот положительных окрашенных клеток CD45 (R3) выполняется точно.

12 Расчет результатов анализа
Расчет концентрации клеток выполняется в рамках анализа данных программным обеспечением, что дает результат определения показателя CD4% и количества клеток CD4+ на 1 мкл образца крови, а также количества лимфоцитов на 1 мкл образца крови после каждого анализа.
Для получения дополнительной информации см. ИЭ цитометра CyFlow™ Counter (CY-S-3022IFUEN).

13 Интерпретация результатов
Вирус иммунодефицита человека (ВИЧ) является одной из основных причин истощения клеток CD4, которая ослабляет иммунную систему. Результаты должны интерпретироваться врачами в сочетании с местными рекомендациями по лечению ВИЧ.

14 Контрольная процедура
Следует проводить надлежащий контроль качества согласно местным и национальным нормативным требованиям.
Существуют различные типы стабилизированного контроля крови образцы доступны на розничном рынке. Не все подходят для каждого потока цитометре. Вы можете узнать, какой контроль крови вы используете на вашем Sysmex представителя. Если критерии приемки не выполнены после измерения подходящей контрольной пробы крови, не приступать к анализу образцов пациента.

15 Рабочие характеристики
15.1 Специфичность
Антитело MEM-241 распознает CD4-антиген, трансмембранный гликопротеин массой 55 кДа, экспрессируемый подгруппой Т-лимфоцитов (Т-клетки «хелперы»), а также моноцитами, тканевыми макрофагами и гранулоцитами. Рабочее совещание по дифференцировочным молекулам клеток человека — HCDM (ранее VIII семинар по дифференцировочным антигенам лейкоцитов человека — HLDA), май 2006 г., Квебек, Канада; код WS M241
Антитело MEM-28 взаимодействует со всеми альтернативными формами человеческого антигена CD45 (общий лейкоцитарный антиген) — одноцепочечного трансмембранного белка типа I массой 180–220 кДа с высоким уровнем экспрессии на поверхности всех клеток гемопоэтического происхождения, за исключением эритроцитов и тромбоцитов. III семинар HLDA; код WS NL 833a.

15.2 Способность обнаружения
Выполнялась оценка для предела холостой пробы (LoB), предела обнаружения (LoD) и предела количественного определения (LoQ) согласно спецификациям, указанным в руководстве CLSI EP17-A2.
LoB: ≤50% of LoD
LoD: ≤20 CD4 клеток/мкл
LoQ: ≤40 CD4 клеток/мкл

15.3 Воспроизводимость
Вариабельность в пределах одного и нескольких анализов:
Значения коэффициента вариации (CV) составляют менее 10 % для количества CD4-клеток, равного или превышающего 200 CD4-Т-клеток на 1 мкл.
Значения коэффициента вариации (CV) для значений CD4% ≥ 25% ниже 10%.
Значения коэффициента вариации (CV) составляют менее 15 % для абсолютного количества CD4-клеток ниже 200 CD4-Т-клеток на 1 мкл.
Значения стандартного отклонения (SD) ≤ 2,5% для значений CD4% ниже 25%.

15.4 Линейность
Диапазон измерений анализа и линейность анализа валидны в пределах следующего диапазона:
абсолютное количество CD4-клеток:40-2500 CD4-Т-клеток на 1 мкл
CD4%: 4 – 60%

15.5 Правильность
Образцы цельной крови с ЭДТА окрашивали с помощью набора CD4% easy count kit и анализировали на цитометре CyFlow™ Counter (CyView™ 2.11). Абсолютное количество и процентное содержание CD4-клеток сравнивали с результатами анализа на цитометре BD FACSCalibur с использованием реагентов BD Tritest CD3/CD4/CD45 и пробирок BD Trucount. Систематическое расхождение между методами составило
-22,56 % (абсолютное количество CD4-клеток) и +2,1 % (CD4%).

16 Ограничения

ВНИМАНИЕ ⚠ *Наборы Sysmex Partec CD4% easy count (05-8405) не были валидированы для использования у пациентов детского и подросткового возраста.*

ВНИМАНИЕ ⚠ *Измерения CD4 и CD4%, сделанные с использованием Sysmex партек испытаний, не должны использоваться наряду с методами обнаружения CD4 и CD4% от других производителей. Используйте тесты только с Sysmex Партек устройствами. Значения, полученные с помощью других инструментов или анализов, не взаимозаменяемы.*

Различные эндогенные и экзогенные вещества были проверены в соответствии с руководством CLSI EP07-A2 на возможность искажения результатов анализа образца крови. В ≥ 18 % случаев гемолиза (вызванного применением протокола, рекомендованного в CLSI EP07-A2, приложение G, G1: метод осмотического шока) наблюдалось искажение результатов. По этой причине не следует использовать гемолизированные образцы. Для определения CD4 и CD4 в целом образцы крови с CyFlow™ Counter анализы, ни один другой антикоагулянт, чем ЭДТА должна быть использована. При наличии дополнительных вопросов о характеристиках продукта обратитесь к местному представителю компании Sysmex.

17 Литература
1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondière, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
2) L. Zijenah, G. Kadzirange, S. Madzime, M. Borok, C. Mudiwa, O. Tobaiwa, M. Mucheche, S. Rusakaniko, D. Katzenstein. (2006). Affordable flow cytometry for enumeration of absolute CD4+ T-lymphocytes to identify subtype C HIV-I infected adults requiring antiretroviral therapy (ART) and monitoring response to ART in a resource-limited setting. Journal of Translational Medicine 4:33
3) H. Karcher, D. Bohning, R. Downing, S. Mashate, G. Harms. (2006). Comparison of two alternative methods for CD4+ T-cell determination (Coulter manual CD4 count and CyFlow) against standard dual platform flow cytometry in Uganda. Clinical Cytometry, 70B (3):163-69
4) S. Kohreanudom, S. Dettraira. (2012). Evaluation of the Partec Single-Platform Volumetric CyFlow® Counter System for Determining Percentage and Absolute Numbers of CD4 T Lymphocytes in HIV/AIDS Thai Patients. Thai AIDS J, 25:1-10
5) D. Wade, P.A. Diaw, G. Daneau, M. Camara, T.N. Dieye, S. Mboup, L. Kestens. (2013). CD4 T-Cell Enumeration in a Field Setting: Evaluation of CyFlow Counter Using the CD4 Easy Count Kit-Dry and Pima CD4 Systems. PLoS ONE, 8(9): e75484

18 Контактная информация производителя
Производитель
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Германия
www.sysmex-partec.com
Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

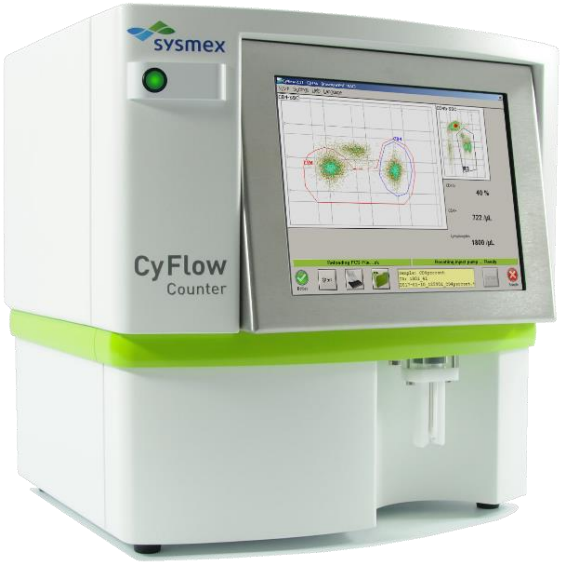
19 Номер редакции и дата выпуска ИЭ
Редакция: ред-07_10-09-2020_CN 1563
Выпущено:: Sysmex Partec GmbH

Паспорт безопасности данного продукта доступен на веб-сайте www.sysmex-partec.com/services.

2.3 CyFlow Counter IFU

CyFlow™ Counter

Instructions for Use



For In Vitro Diagnostic Use

With CyView™ 2.11

Doc. No.: CY-S-3023IFUEN | Rev: 001 | Rev. date: 02-03-2022 | EN | CN 2150

Revision history

Revision 001, Rev. date 02-03-2022

Software: CyView™ 2.11

Initial Version.

Table of contents

List of figures

List of tables

1 Identification

1.1 Product information

Name	CyFlow™ Counter
Software	CyView™ 2.11
Ref. No.	CY-S-3023
UDI-DI	04250878904856

1.2 Intended purpose

IVD

The CyFlow™ Counter as well as the herein stated accessories, reagents and kits are intended for in vitro diagnostic use only.

The CyFlow™ Counter is intended as aid to diagnosis of immune and clinical status of patients and for monitoring, initiation or follow-up of treatment for people living with HIV.

The CyFlow™ Counter is a manual, quantitative clinical flow cytometer designed for the determination of the absolute number of CD4+ T lymphocytes and the percentage of CD4+ cells in lymphocytes in human EDTA venous whole blood samples.

The use of the CyFlow™ Counter is limited to healthcare professionals, trained by staff of Sysmex Partec GmbH or authorized distributors.

1.3 Conformity

CE

The system described in this manual is marked with a CE mark which confirms the compliance with the essential requirements of the following European Directives:

- Regulation (EU) 2017/746 on in vitro diagnostic medical devices
- 2011/65/EU on the restriction of the use of certain hazardous substances in electrical and electronic equipment

1.4 Manufacturer

Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz
Germany

Phone +49 3581 8746-0
Fax +49 3581 8746-70
Mail info@sysmex-partec.com
www.sysmex-partec.com/

1.5 Sysmex representatives

Find address and contact data of your local Sysmex representative also at www.sysmex-partec.com/.

Europe, Middle East and Africa	Sysmex Europe GmbH Bornbarch 1, 22848 Norderstedt, Germany Phone: +49 40 5 27 26-0 Fax: +49 40 5 27 26-100
Americas	Sysmex America, Inc. 577 Aptakisic Road, Lincolnshire, IL 60069, U.S.A. Phone: +1-224-543-9500 Fax: +1-224-543-9505
Asia Pacific	Sysmex Asia Pacific Pte Ltd. 9 Tampines Grande #06-18, Singapore 528735 Phone: +65-6221-3629 Fax: +65-6221-3687
China	Sysmex Shanghai Ltd. 9th Floor, AZIA Center 1233, Lijuazui Ring Road, Shanghai 200120, PRC Phone: +86-21-20524-666 Fax: +86-021-20524-665
Japan	Sysmex Corporation 1-5-1 Wakinohama-kaigandori, Chuo-ku, Kobe, Hyogo 651-0073, Japan Phone: +81-78-265-0500

1.6 Authorised distributors

To receive an overview of authorised distributors nearby, please contact your local Sysmex representative.

2 Introduction

These Instructions for Use will help you to operate your CyFlow™ Counter device.

Please read these instructions carefully before operating the device. Keep the Instructions for Use for future reference.

If you have any questions about the content of the Instructions for Use or operating the device, please contact your local Sysmex representative.

Ordering supplies and parts

To order supplies or replacement parts, please contact your local Sysmex representative.

Service and maintenance

For service and preventive maintenance requests, please contact your local Sysmex representative.

Training courses

For instructional, qualification and training requests, please contact your local Sysmex representative.

For further information on the Sysmex representatives, see section “1.5 Sysmex representatives”.

2.1 General information

Ensure that the following conditions are met when working with the CyFlow™ Counter:

- Read the associated labelling carefully before using the device. This includes all provided labelling of reagents, disposables and consumables used with this device.
- Report any recurring faults or problems to your local Sysmex representative or an authorised distributor.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user is located.

In any case of request for service, complaints or other questions regarding the performance or quality of the product, please contact your local Sysmex representative.

2.2 About these Instructions for Use

While we have taken great effort to ensure that the information in this document is correct at the time of publishing, it may be necessary to update the information as a result of product surveillance activities and publish a new version of the instructions for use.

For any requests regarding the latest revision of the instructions for use, please contact your local Sysmex representative.

2.2.1 Typographical conventions

To ease the reading flow, the following kinds of text highlights are used in this manual.

Table 1: Text highlights

Style	Meaning
[Brackets]	Describes interactive software elements like buttons. Example: Press the [*Start*] button.
Italics	Indicates software elements like menu names, dialog box names, options, and messages.
"Quotes"	Quoted text describes reference sources, cross references to other sections or references to software messages. Examples: Please refer to section "8 Software" for further information. Wait until message "*Cleaning …done*" is displayed in the status bar.

2.2.2 Trademarks

- Sysmex is a registered trademark of SYSMEX CORPORATION.
- Other company names and product names in this manual are the registered trademarks or trademarks of their respective owners.

A not explicitly indicated trademark in this manual does not authorise its use. ™ and ® are not explicitly indicated in the manuals.

2.3 Limitation of liability and warranty

No warranty or liability for any damage or personal injury can be claimed nor protection be guaranteed in the following cases:

- Failure to follow the instructions for use
- Use of the device outside the intended use and specifications described in the Instructions for Use or the Service Manual
- Changes made to the device outside the limitations described in the Instructions for Use or the Service Manual
- Failure to comply with the specified maintenance intervals
- Use of accessories other than approved by Sysmex Partec
- Use of spare parts other than approved by Sysmex Partec

For any warranty claims please contact your local Sysmex representative.

3 Safety

The instructions for use are part of the product and must be read carefully before use. Keep the instructions for future reference.

The product may only be used as intended, any kind of misuse is not permitted.

3.1 Intended use

The CyFlow™ Counter is intended as an aid to diagnosis of immune and clinical status of patients and for monitoring, initiation or follow-up of treatment for people living with HIV.

The CyFlow™ Counter is a manual, quantitative clinical flow cytometer designed for the determination of the absolute number of CD4+ T lymphocytes and the percentage of CD4+ cells in lymphocytes in human EDTA venous whole blood samples.

- Do not use with liquids or materials other than stated in the documentation provided by the manufacturer.
- Do not use as a personal computer.
- Do not place any objects on top of the device.

Use of the CyFlow™ Counter is limited to qualified service personnel and trained healthcare professionals. Training is provided by Sysmex affiliates or authorised distributors on the handling of the CyFlow Counter and reagent preparation by using the CD4 and CD4% assays. Healthcare professional users must have fundamental knowledge of basic laboratory techniques including pipette skills, safe and proper handling of specimens, reagents as well as basic computer skills.

3.2 Safety conventions

Safety information in these instructions includes warnings that will alert you to certain hazards that may arise when using the device. Hazards are classified into different levels according to their severity and the following signal words are used to make you aware of related risks:

WARNING

Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

CAUTION

Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury.

NOTICE
Indicates a hazardous situation that, if not avoided, may result in damage to the device.

Important information that is not safety-related is indicated as follows:

INFORMATION
Indicates additional information about the device or procedure that might be useful during operation.

3.3 Safety symbols

To make warnings more accessible, the following symbols are used to reference the kind of hazard.

Table 2: Safety symbols

Symbol	Description
	Biological hazards
	Electrical hazards

3.4 General safety precautions

3.4.1 Operating environment

The device is designed for indoor use. Failure to observe the operating conditions can impair the function of the device and adversely affect measured results. Make sure the environmental conditions listed in section “11.1 Environmental conditions” are met. They include but are not limited to the following requirements:

- Place the device on a dry, clean, level surface.
- Protect the device from heat, dirt, dust, smoke or any kind of vibrations.
- Do not expose the device to direct sunlight.
- Do not place any objects on top of the device

Device damage

Operating the device at ambient temperature after it was stored or transported at cold temperature may cause internal condensing and possible damage to the device.

- Allow the device to come to ambient temperature before putting the device into operation.

3.4.2 Personal protective equipment

Operating the device includes the handling of human-sourced materials as well as chemical substances.

- Wear appropriate personal protective equipment, e.g. safety glasses, gloves, or protective clothing to minimize direct exposure to infectious materials or hazardous substances.

3.4.3 Working in a clinical laboratory

A clean and safe laboratory working environment is essential for the proper operation of laboratory instruments, the safe use of laboratory equipment and the determination of reliable results. It is therefore important to know and follow generally accepted rules and principles for working in the laboratory. These rules and principles include but are not limited to the following:

- Always handle and maintain laboratory equipment according to the manufacturer's documentation. E.g. make sure pipette and pipette tips are compatible and fit in size.
- Only use clean, intact laboratory equipment that is fully operational.
- Do not use reagents beyond expiry date.
- Use disposable materials (e.g. sample tubes, pipette tips) only once and discard after use. Do not reuse.

3.4.4 Alterations to the device

Unauthorised alterations to the device or the software may result in risks towards users and the device itself. Handle and maintain the device only as described in these instructions.

3.5 Biological hazards

This product requires the handling of human-sourced material.

Risk of infection

All human-sourced materials and any objects coming into contact with those materials have the potential to transmit infections.

To avoid infection:

- Always wear appropriate personal protective equipment.
- Keep your working area clean and follow procedures for weekly cleaning and disinfection described in this manual.
- Dispose of all materials considered potentially infectious in accordance with local regulations.
- Follow all safety regulations applicable to your institution.

3.6 Chemical safety

Chemical hazard

Operating the device includes the handling of consumables that may contain hazardous chemicals. Contact with skin or eyes may cause irritation, burns or eye damages.

Follow general safety guidelines to minimize chemical-related hazards:

- Before handling chemicals, read and understand product-related Safety Data Sheet (SDS) provided by the manufacturer.
- Always observe product-specific warnings, precautions and handling instructions.
- Minimize exposure to chemicals. Wear appropriate personal protective equipment, e.g. safety glasses, gloves, or protective clothing.
- Regularly check for spills or leaks and follow cleanup procedures according to manufacturer’s instructions.
- Comply with applicable laws and regulations related to the handling, storage and disposal of chemical substances.

Potentially explosive metal azides

Some reagents contain sodium azide as preservative. Copper and lead used in some plumbing systems can react with azides to form explosive salts.

- Dispose of azide-containing materials with large volumes of water to prevent azide build-up.

3.7 Waste handling and disposal

Hazardous waste

Liquid waste from the device may contain hazardous substances. All materials which have been in contact with potentially infectious substances of human origin must be considered biohazardous.

To minimize waste-related hazards:

- Characterise the waste stream of your facility and make sure all waste is disposed of in accordance with applicable regulations.
- Read and understand product-specific Safety Data Sheets (SDSs) and safety precautions provided by the manufacturers of chemical substances used before storing, handling or disposing chemical waste.
- Wear personal protective equipment (e.g. safety glasses, gloves, protective clothing).

3.8 Laser optics

The device is categorised as a class 1 laser product according to EN 60825-1:2014. However, the device is equipped with a class 3b laser. Under normal operating conditions the device housing is closed and the optical system is protected by secured covers. The covers are not intended to be removed by the operator. Only authorised service personnel may install and maintain internal components.

- Do not open the device housing.
- Do not remove laser covers.

3.9 Electrical hazards

The device has been designed, manufactured and tested to conform to safety requirements for electrical equipment for laboratory use.

Please observe the following general requirements when operating the device:

- Use an appropriately grounded electrical outlet of correct voltage.
- Place the device in such a way that power cables are easily accessible to unplug and will not be damaged in any way.
- In case of defect instrument cables, replace them with adequate approved cables. Refer to spare parts list for further information.

Uninterruptible power supply

Power failure or drops in voltage will lead to the device being shut down causing possible data loss.

- It is recommended to use an uninterruptible power supply (UPS).

3.10 Product-related symbols

The following symbols appear on the device, on device packaging or in product labelling.

Symbol	Description
	Manufacturer
	Country of manufacture including the date of manufacture
	In vitro diagnostic medical device
	Serial number
	Reference number
	CE mark
	Consult instructions for use
	Caution
	Biological risks
	Keep away from sunlight
	Temperature limit
	Humidity limitation
	Unique device identifier
	Waste of Electrical and Electronic Equipment
	General electrical data

3.11 Labels used on the device

The following labels are attached to the rear side of the device.

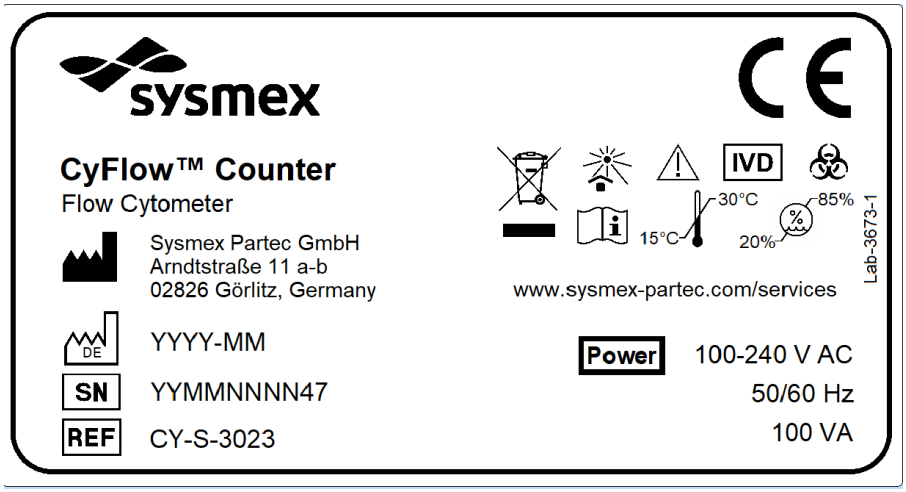


Figure 1: Type plate

Figure 2: Class 1 Laser Product label

Figure 3: Unique device identifier barcode

4 Device components and functions

The CyFlow™ Counter is a fully equipped compact and robust desktop flow cytometer with green laser excitation and three optical parameters (SSC and 2 fluorescence channels). It performs fluorescence analysis and True Volumetric Absolute Counting (TVAC) without the need for reference beads or a hematology analyzer. It represents a reliable solution with variable sample throughput for local health care centers as well as district and regional hospitals.

The CyFlow™ Counter is simple to install and does not need any additional setup time. Results are presented within three minutes as diagrams and as counting results in “cells/µL” blood sample or percentage.

4.1 Device components

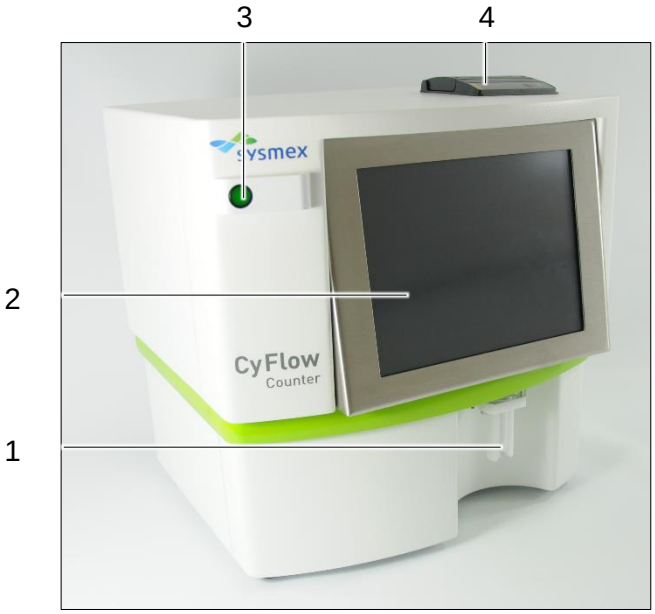


Figure 4: Front view

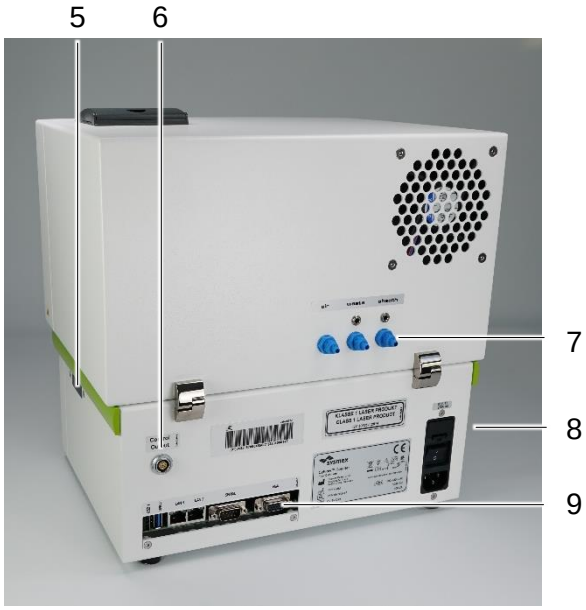


Figure 5: Rear view

1	Sample port	6	Control output for extension
2	Display (Touch-display)	7	Coupling points for fluidics
3	ON/OFF button	8	Power switch, power cord connection and fuse holder
4	Thermal printer	9	Ports for external devices
5	Coupling point for extension		

4.1.1 Sample port

The sample port is the only way to connect sample tubes. It is the point of intake for sample material.

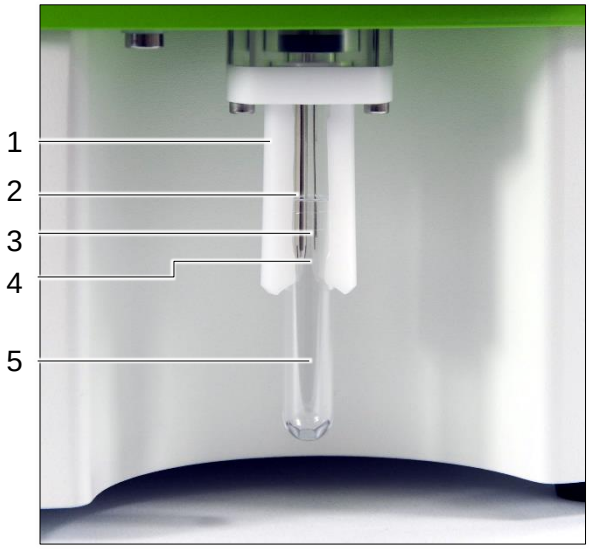


Figure 6: Sample port with sample tube (default condition)

1	Test tube holder	4	Stop electrode
2	Sample port injection needle	5	Sample tube
3	Start electrode		

4.1.2 Thermal printer

INFORMATION
Thermal paper is not suited for long-term storage. For permanent or long-term storage make a copy on regular paper. Please see chapter “8 Maintenance” on how to renew and adjust the printer paper.

The status LED of the printer is an indicator of functionality. Two states of operation are possible:

- Permanent light: Operational
- Blinking light: Error
- None: Off / Error

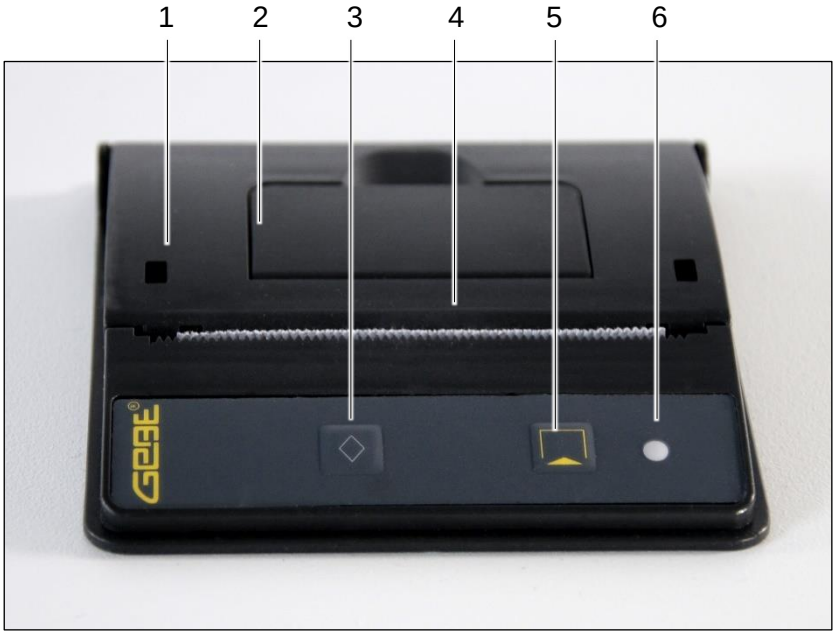


Figure 7: Thermal printer

1	Paper tray cover	4	Paper output
2	Latch	5	Feed button
3	Test print button	6	Status LED

INFORMATION

If the cover of the paper tray is not closed correctly, the print jobs will not be executed but queued. After closing the paper tray correctly, all print jobs in the queue will be processed.

4.1.3 Coupling points for fluidics

The coupling points for fluidics are inlet for Sheath Fluid by the Sheath bottle and air for building the vacuum as well as the outlet for waste.

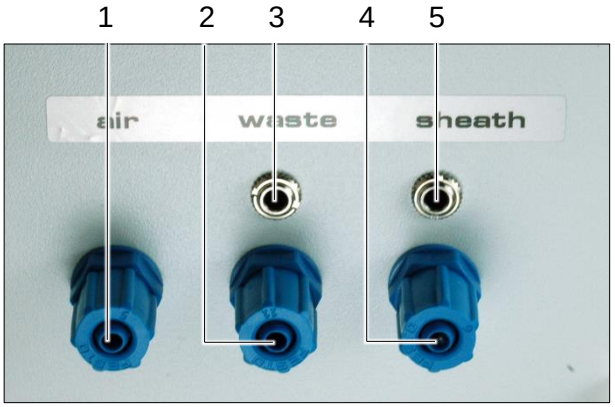


Figure 8: Coupling points for fluidics

1	Air tubing connection	4	Sheath tubing connection
2	Waste tubing connection	5	Sheath sensor cable port
3	Waste sensor cable port		

4.1.4 Fuse holder, main power switch and power inlet socket

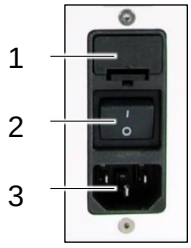


Figure 9: Power supply ports

1	Fuse holder (T 3.15 A / 250 V)	3	Power inlet socket
2	Main power switch		

4.1.5 Ports for external devices

The device has several ports for connection of external devices. None of them are necessary for the intended operation of the device. Any access is restricted to Sysmex authorized service personnel.

NOTICE

System and data modification by unauthorised personnel

Transferring data unauthorised with an external device onto the system may cause damages to system files and stored data.

- Only authorised service personnel are allowed to transfer data onto the system via an external device.
- Do not manipulate or delete data and files without permission from the manufacturer or from personnel authorised by the manufacturer.

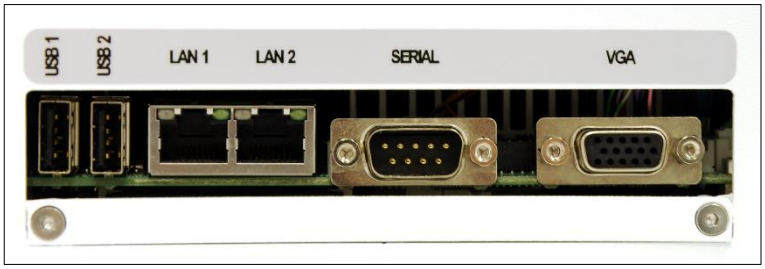


Figure 10: Ports for external devices

USB 1	USB 2.0 port	*LAN 2*	Ethernet port
USB 2	USB 3.0 port	*SERIAL*	RS 232 port
LAN 1	Ethernet port	*VGA*	VGA port

4.1.6 Sheath bottle

The bottle is coated with a protective layer and can bear up to 1000 mL contents. The scale on the bottle displays the content value in mL.

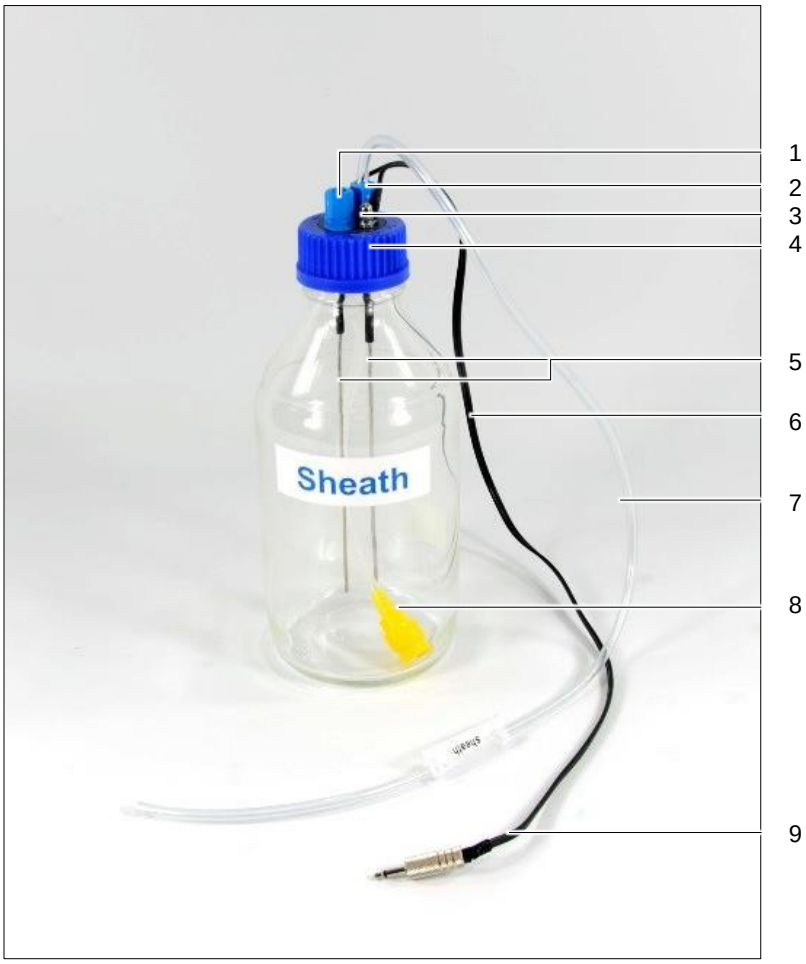


Figure 11: Sheath Fluid bottle assembled (empty)

1	Tubing connection (relief)	6	Sheath sensor cable
2	Tubing connection Sheath Fluid	7	Sheath Fluid tubing
3	Sensor cable connection for the Sheath bottle	8	Inline filter (with internal Sheath Fluid tubing)
4	Cap Sheath bottle	9	Sheath sensor cable plug
5	Electrodes		

4.1.7 Waste bottle

The bottle is coated with a protective layer and can bear up to 1000 mL contents. The scale on the bottle displays the content value in mL.

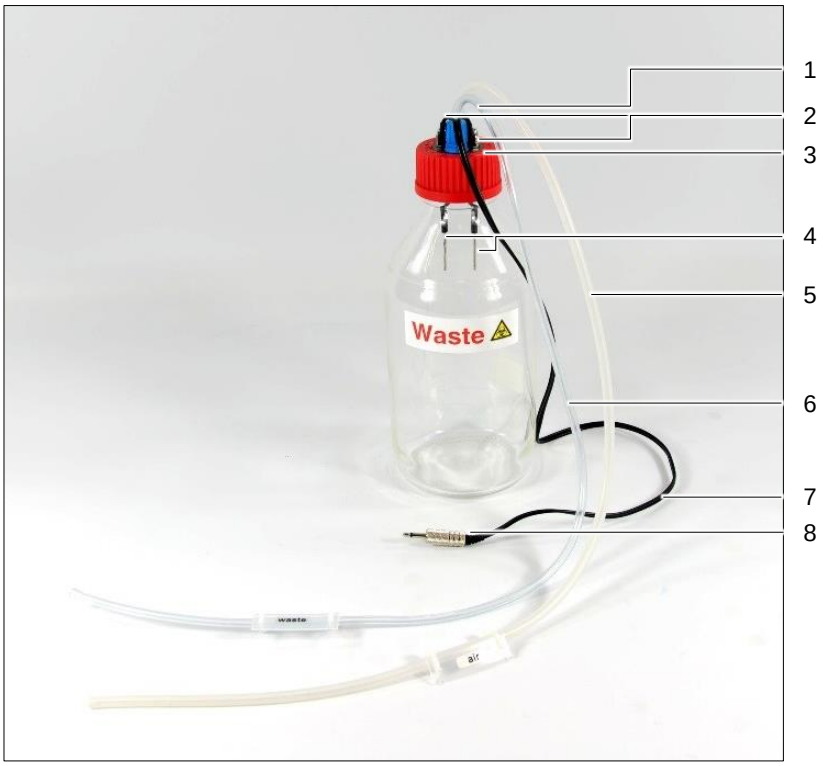


Figure 12: Waste Fluid bottle complete (empty)

1	Tubing connection waste	5	Air tubing
2	Sensor cable connection for the waste bottle	6	Waste tubing
3	Cap waste bottle	7	Waste sensor cable
4	Electrodes	8	Waste sensor cable plug

4.1.8 Sheath Fluid and tap

The Sheath Fluid container 5 L is only usable with an appropriate tap affixed. Please see the labelling of the Sheath Fluid container for instructions. The tap is provided separately.

Figure 13: Sheath Fluid container (exemplary, left) and tap (right)

1	Sheath Fluid container	2	Tap

4.2 Reagents, consumables and spare parts

For further information on our range of products and additional parts, visit our website www.sysmex-partec.com.

Table 3: Reagents and kits

Item	Content	Ref. No.	
		IVD-D	IVD-R
Sheath Fluid	5 L	04-4007	04-4016
Tap for Sheath Fluid	1 piece	04-4006	04-4006
Cleaning Solution	250 mL	04-4009	04-4017
Decontamination Solution	250 mL	04-4010	04-4018
Hypochlorite Solution	250 mL	04-4012	04-4019
Count Check Beads green	25 mL	05-4011	05-4026

Item	Content	Ref. No.	
		IVD-D	IVD-R
CD4 easy count kit	100 tests	05-8401	05-8410
CD4% easy count kit	100 tests	05-8405	05-8411

Table 4: Consumables

Item	Content	Ref. No.
Sample tubes 3.5 mL (single use)	500 pieces	04-2000
Sample tube rack	1 piece	04-2000-02
Inline filter for Sheath container	10 pieces	04-004-1000
Thermal printer paper roll	5 pieces	04-4000

Table 5: Spare parts

Item	Content	Ref. No.
Spare Fuses T 3.15 A	5 pieces	06-7-8005
Sheath Bottle 1 L laminated	1 piece	04-200-1071
Waste Bottle 1 L NO.03	1 piece	04-200-1059
Cap 1 L Waste Bottle NO.04	1 piece (incl. level sensor)	04-200-1043
Cap 1 L Sheath Bottle NO.02	1 piece (incl. level sensor)	04-200-1044
Instrument cable Type E+F	1 piece	06-300-1041

4.3 Flow cytometer functionality

4.3.1 Flow cytometry principle

In general flow cytometers consist of:

- a vacuum-based fluidic system to transport the sample through the device and the measuring chamber, the flow cuvette,
- a light source based optical system to generate and collect the light signals from the particles inside the flow cuvette,
- optical detectors and appropriate electronics to pick up the optical signals and transfer them in electrical and later on in digital signals and
- a computer system with a software to operate the system and to calculate and visualize the results.

Flow cytometry detects signals individually for each particle floating in a sample flow. The CyFlow™ Counter uses this principle to detect fluorescent stained blood cells.

These particles can be analysed and counted in general but also depending on subpopulations. Results can be calculated as fractions of different cell populations.

For determination of concentration, detected and counted particles must refer to a certain volume. The single-platform technique by Sysmex Partec measures the volume of the sample simultaneously. The cell concentration is calculated with the following formula:

$$c = \frac{N}{V}$$

Formula 1: Cell concentration formula

Cell concentration (*c*) is determined by precisely counting the number of cells (*N*) suspended in a mechanically defined volume (*V*).

Please refer to complying specialist literature[1] for further information on basic principles of flow cytometry.

4.3.2 Fluidic system and theory of operation

The fluidic system of the CyFlow™ Counter is served by a vacuum. This vacuum is applied to the waste bottle. It is precisely controlled and monitored by the electronics.

By this vacuum the so called Sheath Fluid is pulled through the fluidic system of the device. The sheath fluid conducts the hydrodynamic focusing inside the flow cuvette.

The sample is injected into this sheath flow directly below the flow cuvette. The effect of hydrodynamic focusing centers the sample in a core stream in the flow cuvette. Simultaneously the cells are separated ideally to a single line. In the flow cuvette the cells are then passing the interrogation zone with the light source.

The sample feeding of the device takes place at the sample port, where the sample tube will be fixed tightly. A syringe pump pushes air into the sample tube, which forces the

[1] Example: Howard M. Shapiro, Practical Flow Cytometry, John Wiley & Sons Inc., Hoboken, New Jersey, 2003

sample to vanish through the aspiration needle into the device and will be incorporated into the sheath flow.

The syringe pump is computer controlled and therefore the speed and displaced volume are determined.

Volume determination is provided by two electrodes, which are implemented in the sample port, in direct neighborhood to the sample aspiration needle. A start signal is send to the computer while the fluidic level inside the sample tube is passing the first electrode, and the stop signal follows after passing the second electrode. The volume between the two electrode signals is precisely known. This technique is known as TVAC (true volumetric absolute counting).

Based on the cell concentration formula (see above) the concentration is then calculated.

Furthermore the fluidic system is able to clean the system including the flow cuvette and the sample port backwards and forwards either by sheath fluid or by cleaning fluids provided at the sample port.

4.3.3 Optical system and theory of operation

Fluorescent based flow cytometry is depending on optical components. Basically there are two groups – the illumination or excitation part and the collection of emission part. In between there is the flow cuvette as the interrogation zone.

The CyFlow™ Counter uses a solid state laser for excitation. The laser beam is shaped and focused into the flow cuvette by appropriate laser optics. The position of the laser can be adjusted to align the laser exactly into the flow channel of the flow cuvette.

On the emission side the CyFlow™ Counter has three detectors. These are one optical parameter Side scattered light (SSC) and two fluorescent parameters (FL2 and FL3). The SSC detects the scattered light from the laser beam hidden cell which is related to the shape and structure of the cell. The fluorescent parameters are detecting the fluorescent light depending on the staining of the cells. In the CyFlow™ Counter this is optimized to the dyes used in the CD4 easy count kit and CD4% easy count kit.

The SSC is connected via fiber optic and a photo multiplier tube (PMT) plugged to the electronics. The fluorescent channels are each consisting of an align free collection optics, the appropriate optical filter and a PMT.

4.3.4 Electronics and signal processing

In the PMT, optical signals are converted into electrical signals. These signals are amplified and converted into digital signals by an analog-to-digital converter (ADC). In case of the CyFlow™ Counter the ADC has a resolution of 16 bits respective 65535 discrete levels. For each detector the peak height (peak maximum) of the signal is acquired.

At least one channel is selected as the so called trigger channel. A signal need to appear in this channel to start (trigger) the data acquisition of all other channels. For CD4 absolute the trigger channel is set to FL2, for CD4% the trigger channel is set to FL3. This trigger channel is fixed for the application.

In the trigger channel a threshold is applied to exclude signals from debris or electronic noise from being acquired. This threshold is predefined for each application during setup.

4.3.5 Result presentation and analysis

After acquisition, data are transferred to the user interface software. The data are displayed as intensity. Distribution of intensity signals can be shown either for one channel (histogram plot) or for two channels (dot plot). It is common in flow cytometry to use a logarithmic scale for graphical presentation. This logarithmic calculation is done purely in software. For CD4 absolute and CD4% measurements only the logarithmic presentation is used.

To analyze the data, regions can be drawn. In histograms these are range, in dot plot these are polygons. The number of cells falling into each region can be determined by the software and thereby the concentration and ratio of the cell populations can be calculated and presented as results.

5 Installation

The CyFlow™ Counter was tested before shipping. Upon receipt of the device, an authorised service technician unpacks, positions and installs the device.

5.1 Pre-installation information

5.1.1 Transport material

Keep all transport and protective materials for future use and store them in a dry place, in case transport or storage for a longer period becomes necessary.

The following transport securing materials are used to avoid damage to specific device components. Make sure to keep these materials for future use.

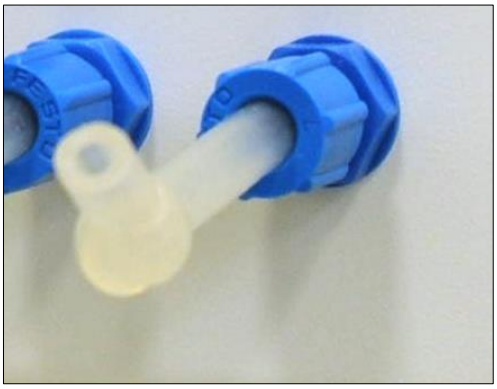


Figure 14: Knotted tubings to secure the fluid connections

Figure 15: Inserted sample tube to secure the sample port components

5.1.2 Installation site

The installation site must meet the requirements for the operating environment and electrical specifications as described in chapter “11 Technical data”.

It is recommended to use an uninterruptible power supply if frequent power failures are expectable at the installation site.

5.2 Scope of delivery

The following items are in the scope of delivery:

- CyFlow™ Counter
- Sheath and waste bottle (empty and sealed)
- Instrument cable (E+F)
- Bottle cap set
- Inline filter
- Pen for touchscreen (2x)
- Protective cover
- Documents
  - End User License Agreement
  - Instructions for Use

5.3 Installation

Initial installation of the CyFlow™ Counter is performed by authorized service personnel. This includes adjusting the device and registering the clinic ID as well as the lab ID.

5.4 Relocating the device

Sometimes it may be necessary to relocate the device, for example in case of cleaning work or fixing the environment in a laboratory.

Take the following general precautions:

- Always carry the CyFlow™ Counter in upright position.
- Do not touch the electrodes of the sample port.

WARNING

Protective earthing

The electrical protection for the operator is not realised, if the device is plugged into a non-groundet electrical outlet.

- Connect the device to a grounded electrical outlet.

WARNING

Risk of infection

All human-sourced materials and any objects coming into contact with those materials have the potential to transmit infections.

- Always wear appropriate personal protective equipment.
- Clean and disinfect spills immediately.
- Follow all safety regulations applicable to your institution.

CAUTION

Negative effects on measurement results through vibration

Vibrations in the operating environment may interfere with the optics alignment. This may result in false high or low CD4 counts and have a negative impact on treatment decisions.

- Do not expose the device to any kind of vibration.

INFORMATION

When screwing or unscrewing a bottle and its respective cap, it is advantageous to twist the bottle, to keep the tubing unbent.

5.4.1 Disconnecting

Before disconnecting tubing connectors and sensor cables, perform shutdown procedure as described in section “6.7 Shutdown”.

Required material

Item	Quantity
Container for collecting residual liquids	1 piece
Tissue or cloth to catch liquid	2 pieces

Prerequisite

- Device is powered off
- Wear appropriate personal protective equipment

Procedure

1. Unplug the power cord.
2. Disconnect the waste tubing.
   Catch any liquid with a cloth.
3. Disconnect the air tubing.
4. Disconnect the Sheath tubing.
   Catch any liquid with a cloth.

5. Disconnect the sensor cable plug Sheath Fluid.
6. Disconnect the sensor cable plug waste.
7. Empty the Sheath bottle properly. See chapter “8.2.4 Empty and refill Sheath Fluid”.
8. Empty the waste bottle properly. See chapter “8.2.3 Empty liquid waste”.

Result

You can now move the device to another place. In case the device will be idle for a time between two and six days, cover it with the protective cover. Weekly cleaning is required latest after 7 days. Refer to section “6.7.4 Long-term shutdown and storage” if longer storage of the device is needed.

5.4.2 Connecting

WARNING

Wrong caps on bottles

Mixing up the bottle caps may result in biohazardous waste being pulled into the device. This may result in contamination of the Sheath Fluid tubing.

- Do not screw the waste bottle cap on the Sheath bottle.
- Do not screw the Sheath bottle cap on the waste bottle.

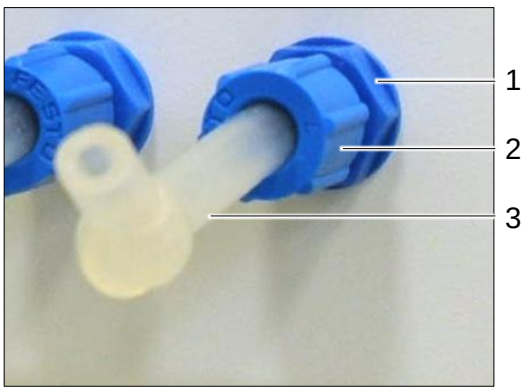


Figure 16: Fluid connection

1	Fluid connection	3	Tubing
2	Fluid connection cap		

Prerequisite

- Device, Sheath bottle and waste bottle are placed on an even, solid, dry and clean surface
- Sheath bottle and waste bottle must be placed at the same height level as the device
- Wear appropriate personal protective equipment

Procedure

1. Assemble the Sheath bottle by screwing the Sheath bottle cap on the filled Sheath bottle.
2. Assemble the waste bottle by screwing the waste bottle cap on the waste bottle.
3. Connect the Sheath sensor cable plug to the Sheath sensor cable port.
4. Unscrew the fluid connection caps (if not already done).
5. Arrange each fluid connection cap on its respective tubing. The cap must be reattachable to the fluid connection.
6. Connect the Sheath Fluid tubing of the bottle cap to the Sheath Fluid connection.
7. Connect the waste sensor cable plug to the waste sensor cable port.
8. Connect the waste fluid tubing of the bottle cap to the waste fluid connection.
9. Connect the air tubing of the cap to the air connection.
10. Connect the power cord to the power inlet socket.
11. Plug the power cord to a mains socket or if applicable plug the power cord to an uninterruptable power supply.

Result

You can now proceed to refill the Sheath bottle. Refer to section “8.2.4 Empty and refill Sheath Fluid” for further information.

6 Operation

This section describes standard procedures for operating the device.

NOTICE

Possible damage to the device

Failure to follow these instructions for use may result in damages to the device and may adversely affect measurement results.

- Read and follow instructions carefully, including section “7 Software”.

The CyFlow™ Counter operates under software control. Processing is automated via task-related configuration files. The following table lists individual processes and associated configurations.

Table 6: Operating procedures and configurations

Operating procedure	Configuration
Start-up	
1. Booting	-
2. Cleaning	Cleaning.scr
Internal Quality Check	CountCheckBeadsGreen.scr
Standard operations	
1. CD4 measurement	CD4.scr
2. CD4% measurement	CD4percent.scr
Emergency Cleaning	EmergencyCleaning.scr
Shutdown	
1. Cleaning	Cleaning.scr
2. Shutting down	-
3. Power off	-

6.1 Scope of operation

INFORMATION
The herein recommended items are necessary to operate the CyFlow™ Counter as intended by Sysmex Partec. For further information on how to obtain these items, please see section “4.2 Reagents, consumables and spare parts”.

The following items are recommended for operating the CyFlow™ Counter. The table also shows which of the items are available through Sysmex and distributors authorised by Sysmex.

Table 7: Scope of operation availability matrix

Item	Scope of delivery	Sysmex order item
CyFlow™ Counter (device only)	✓	✓
Instructions for Use	✓	✓
Reagents and kits		
Sheath Fluid		✓
Cleaning Solution		✓
Decontamination Solution		✓
Hypochlorite Solution		✓
Count Check Beads green		✓
CD4 easy count kit		✓
CD4% easy count kit		✓
Disposables		
Pipette tips 50-1000 µL		
Pipette tips 2-200 µL		
Sample tubes 3.5 mL		✓

Item	Scope of delivery	Sysmex order item
Consumables		
Sample tube rack		✓
Pipette variable 100-1000 µL		
Pipette fix 20 µL		
Pipette fix 10 µL		
Spare Parts		
Sheath bottle	✓	✓
Waste bottle	✓	✓
Waste bottle cap	✓	✓
Sheath Fluid bottle cap	✓	✓

6.2 Start-up preparation

NOTICE

Inline filter air bubbles

Air bubbles might aggregate underneath the inline filter after closing the Sheath bottle with its cap. Air bubbles cause erroneous data and hinder correct operation.

- Remove air bubbles by moving the Sheath Fluid bottle cap gently up and down while keeping the inline filter completely covered in Sheath Fluid of the bottle.

Before operating the device, make sure that the following conditions are met:

- Sheath bottle and waste bottle are clean and placed at the same height level as the device
- Sheath bottle is filled with Sheath Fluid, the electrodes must be covered
- inline filter is in the Sheath bottle tubing and unobstructed
- inline filter is free of air bubbles
- waste bottle is empty, the electrodes must be uncovered
- Sheath bottle and waste bottle are closed, each with its respective cap

- all cables are connected
- all tubings are connected
- cables and tubings from and to the device are unbent
- the ventilation grid at the rear side is unobstructed
- avoid exposure of the device to smoke, dust, vibrations, direct sunlight and heat

6.3 Start-up

Start-up must be performed before the Internal Quality Check (IQC) and before any blood sample measurement.

6.3.1 Booting

INFORMATION
For devices provided with less than 200 V, switching on the main power switch automatically starts the device.

1. Press the main power switch on the rear side.
2. Start the CyFlow™ Counter by pressing the ON/OFF button.
3. The system is booting until the operating system is ready.
4. CyView™ runs.

Continue with “Cleaning”.

6.3.2 Cleaning

The *Cleaning* configuration is executed as first configuration when starting-up, please see chapter “7 Software” additionally to this chapter.

Required material

Item	Quantity
Cleaning Solution	1.6 mL
Sheath Fluid	1.6 mL
Sample tube 3.5 mL	2 pieces
Pipette tips 50-1000 µL	2 pieces
Pipette variable 100-1000 µL	1 piece

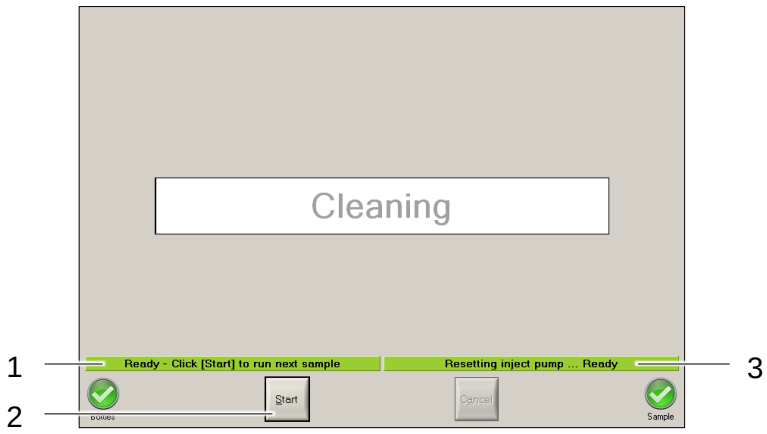


Figure 17: Cleaning

1	Device status bar	3	Progress status bar
2	*[Start]* button		

Procedure

1. Select *Settings* > *Load Configuration* > *Cleaning.scr* from the drop-down menu.
2. Plug a sample tube with 1.6 mL Cleaning Solution gently into the sample port.
3. Press [*Start*] button.
4. Wait until message "*Cleaning …done*" and message "*Setting Inject pump…Ready*" is displayed in the status bars.
   The sample status indicator shows the "Error" symbol.
5. Plug a sample tube with 1.6 mL Sheath Fluid gently into the sample port.
6. Press the [*Start*] button.
7. Wait until message "*Cleaning …done*" and message "*Setting Inject pump…Ready*" is displayed in the status bars.
   The sample status indicator shows the "Error" symbol.

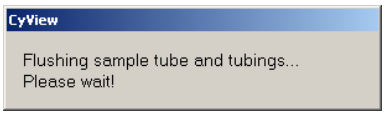


Figure 18: Flushing before Cleaning is done

Figure 19: Cleaning is done

Continue with Internal Quality Check.

6.4 Internal Quality Check

The Internal Quality Check (IQC) is intended to verify device function. This is done by analysing the Count Check Beads green (CCBg) concentration to confirm volumetric counting precision. For further information, please see chapter “7 Software” additionally to this chapter.

The following parameters form the basis for a correct analysis of the Count Check Beads green concentration:

- Position of peak: confirms laser power, gain value settings, optical alignment
- Peak width: confirms optical alignment, working fluidics

6.4.1 CCBg measurement

Required material

Item	Quantity
Count Check Beads green	850 µL
Sample tube 3.5 mL	1 piece
Pipette tips 50-1000 µL	1 piece
Pipette variable 100-1000 µL	1 piece

Prepare sample

1. Make sure the Count Check Beads green bottle is closed.
2. Shake the Count Check Beads green bottle thoroughly for about 15 seconds.
3. Use a pipette to transfer 850 µL Count Check Beads green into a clean sample tube. Discard pipette tip.

Measurement

4. Select *Settings* > *Load Configuration* > *CountCheckBeadsGreen.scr* from the drop-down menu
5. Plug the sample tube gently into the sample port.
6. Press the [*Start*] button.
7. Enter a sample ID (max. 50 characters) via the on-screen keyboard. Confirm by pressing the [*Enter*] button.
8. Wait until message “*Cleaning …done*” and “*Setting Inject pump…Ready*” is displayed in the status bars.
   The sample status indicator shows the “Error” symbol.

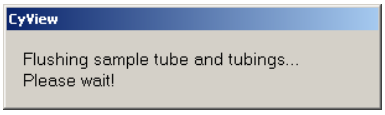


Figure 20: Flushing before Count Check Beads green is done

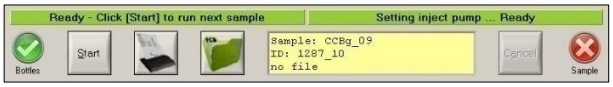


Figure 21: Count Check Beads green is done

Cancelling a measurement

Cancel a measurement by pressing the [Cancel] button, while running a measurement.

The following dialogue offers two options, to either

- Press [*Yes*] and flush the sample tube automatically now.
- Press [*No*] and flush the sample tube manually at a later time.

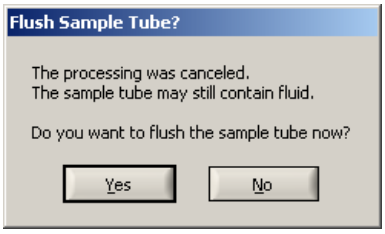


Figure 22: Flush Sample Tube

Measurement diagram

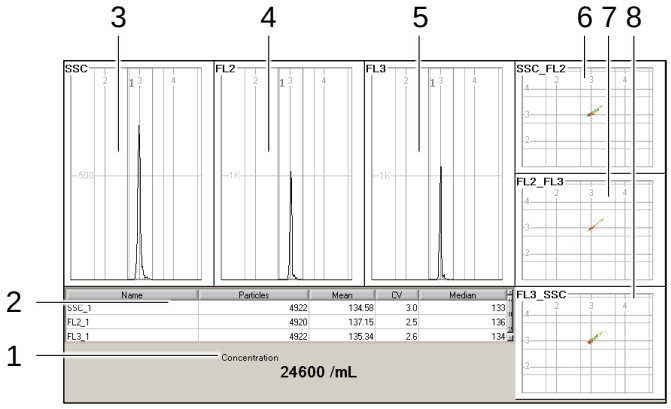


Figure 23: CCBg measurement (exemplary)

1	Concentration result (count/mL)	5	“FL3” histogram
2	Detection values	6	“SSC_FL2” dot plot
3	“SSC” histogram	7	“FL2_FL3” dot plot
4	“FL2” histogram	8	“FL3_SSC” dot plot

6.4.2 CCBg measurement post-treatment

Result interpretation

Compare the concentration result (count/mL) with the lot-specific concentration stated on the label of the used Count Check Beads green bottle. The IQC is passed if the measured value is within or exactly ± 10% of the lot-specific concentration value stated on the CCBg bottle.

If IQC did not pass, please see chapter “9.2 Internal Quality Check out of range”.

If IQC is passed, the device is ready for standard operations.

Printout

CCBg measurement results can be printed out.

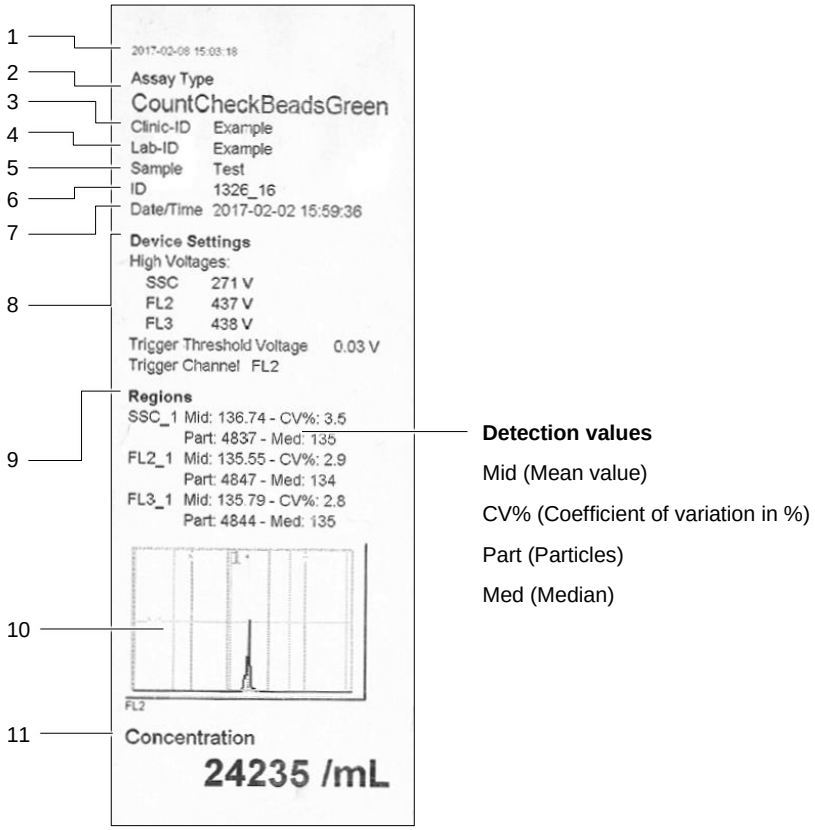


Figure 24: CCBg printout (exemplary)

1	Timestamp of printout (YYYY-MM-DD hour:minute:second)	7	Measurement date (YYYY-MM-DD hour:minute:second)
2	Assay type (CountCheckBeadsGreen configuration)	8	Device Settings
3	Clinic-ID (generated at commissioning)	9	Regions (detailed region results)
4	Lab-ID (generated at commissioning)	10	Diagram with caption (FL2)
5	Sample ID (user-defined)	11	Concentration result (CCBg/mL)
6	Measurement ID (automatically generated)		

Table 8: IQC detection values

Detection value	Description
Name	Name of detector (e.g. “SSC_1” for SSC photomultiplier tube)
Particles	Number of counted particles for each detector (absolute)
Mean	Mean intensity value for all measured particles (statistical)
CV	Coefficient of variation in %, which shows the variability in relation to a mean value
Median	Intensity value of the most intermediate particle, depending on the most and least intense particle measured (statistical)

6.5 Sample handling

Observe the following guidelines when handling samples:

- Use human venous whole blood collected in EDTA (anticoagulant, K2 or K3) tubes.
- Invert the closed EDTA tube containing the whole blood specimen 8-10 times prior to preparation to ensure a homogeneous mixture.
- For best results, use the whole blood specimen in combination with CD4 easy count kit or CD4% easy count kit within 6 hours after blood collection.
- If necessary, the whole blood specimen can be stored in a refrigerator (2-8 °C, non-freezing) for up to 24 hours. Discard samples that have exceeded the specified storage period or that were stored under inappropriate conditons.

6.6 Standard operations

WARNING

Disease progression

Users might perform a measurement under wrong environmental influences. Failure to observe the operating conditions can impair the function of the device and adversely affect measured results

- Observe the environmental conditions listed in section “11.1 Environmental conditions” when operating the device.

WARNING

Interchangeability of measurement results

CD4 and CD4% concentrations measured with the CyFlow Counter and Sysmex Partec assays may differ from concentrations obtained from other manufacturers' methods of determining CD4 and CD4%. Patients monitoring based on CD4 and CD4% values measured with different technologies may result in incorrect treatment decisions.

- Results must not be used interchangeably.

During the staining procedure with the CD4 easy count kit and the CD4% easy count kit whole blood specimen are diluted by a factor of 42. The CyView™ configurations "CD4" and “CD4percent” already take this dilution factor into account and display the number of $CD4^+$ T cells and $CD4^-$ lymphocytes per µL of undiluted blood in the respective “Result” area.

Dilution factor for CD4 easy count kit:

$$\frac{20\ \mu L\ blood + 20\ \mu L\ CD4\ mAb\ PE + 800\ \mu L\ no\ lyse\ buffer}{20\ \mu L\ blood} = 42$$

Dilution factor for CD4% easy count kit:

$$\frac{20\ \mu L\ blood + 10\ \mu L\ CD4\ mAb\ PE + 10\ \mu L\ CD45\ mAb\ PE\text{-}Cy5 + 400\ \mu L\ buffer\ 1 + 400\ \mu L\ buffer\ 2}{20\ \mu L\ blood} = 42$$

6.6.1 CD4 measurement

WARNING

Risk of infection

All human-sourced materials and any objects coming into contact with those materials have the potential to transmit infections.

- Always wear appropriate personal protective equipment.
- Clean and disinfect spills immediately.
- Follow all safety regulations applicable to your institution.

NOTICE

Clotted blood samples

Clotted whole blood samples may compromise measurement results and cause clogging of the fluidic system.

- Do not use clotted blood samples.

To understand the software interface and to use the software correctly, please also see chapter “7 Software”.

Required material

Item	Quantity
EDTA whole blood	1 tube
CD4 easy count kit - CD4 mAb PE - no lyse buffer	1 kit
Sample tube 3.5 mL	1 piece
Pipette tips 50-1000 µL	1 piece
Pipette tips 2-200 µL	2 pieces
Pipette variable 100-1000 µL	1 piece
Pipette fix 20 µL	1 piece

CD4 sample preparation

- Invert the blood sample in the EDTA blood collection tube gently 8 to 10 times.
- Pipette 20 µL EDTA whole blood sample to the bottom of an unused sample tube. Avoid leaving a trail of blood from the pipette tip on the inner tube wall. Discard the pipette tip.
- Add 20 µL CD4 mAb PE directly into the blood sample and mix it gently. Avoid leaving a trail of blood from the pipette tip on the inner tube wall. Discard the pipette tip.
- Incubate the mixture for a minimum of 15 minutes and a maximum of 30 minutes at 15 – 30 °C in the dark.
- Add 800 µL no lyse buffer and vortex briefly or mix the sample by pipetting up and down. Discard the pipette tip.
- Vortex briefly or mix the sample by pipetting up and down before analysing the sample.

INFORMATION

After addition of no lyse buffer samples can be stored for up to 2 hours at 2-8 °C in the dark. Do not use samples that have exceeded storage time or that have not been prepared according to the instructions.

See also the instructions for use of the CD4 easy count kit.

Measurement

1. Select *Settings* > *Load Configuration* > *CD4.scr* from the drop-down menu.
2. Plug the sample tube with the prepared sample gently into the sample port.
3. Press the [*Start*] button.
4. Enter a sample ID (max. 50 characters) via the on-screen keyboard. Confirm by pressing the [*Enter*] button.
5. The ongoing step is displayed in the progress status bar.

The instrument performs a series of steps in the following sequence:

a) Prerun: Creates a temporary, fast sample flow.
b) Measure: The laminar flow is established until TVAC starts. Particles are detected.
c) Level Counting: Particles in a certain volume are detected and counted.
d) Flush: An internal cleaning cycle is performed.

6. Wait until message "*Cleaning …done*" and "*Setting Inject pump…Ready*" is displayed in the status bars.
   The sample status indicator shows the "Error" symbol.

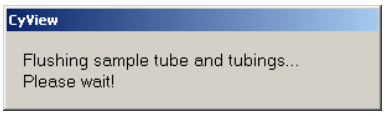


Figure 25: Flushing before CD4 measurement is done

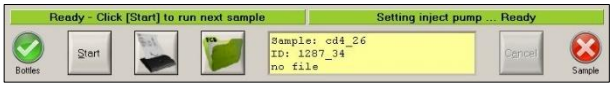


Figure 26: CD4 measurement is done

Measurement diagram

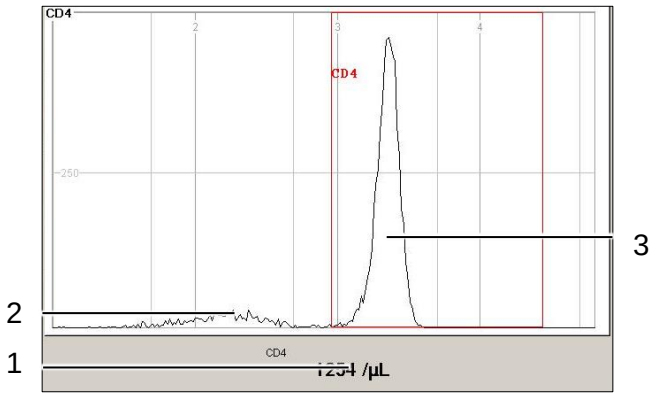


Figure 27: Results of CD4 measurement (example)

1	$CD4^+$ T cells result (cells/µL)	3	$CD4^+$ T cell peak, gated by CD4 gate
2	Monocyte peak		

Cancelling a measurement

Cancel a measurement by pressing the [Cancel] button, while running a measurement.

The following dialogue gives you two options:

- Press [*Yes*] and flush the sample tube automatically now.
- Press [*No*] and flush the sample tube manually at a later time.

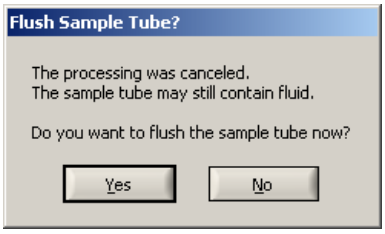


Figure 28: Flush Sample Tube

6.6.2 CD4 measurement post-treatment

Before saving or printing measurement results, verify correct placement of cell populations and debris within the measurement diagram by visual inspection. Only CD4+ T cells must be inside the “CD4” gate. Monocytes and if present debris must be outside “CD4” gate. Otherwise, gate “CD4” needs to be adjusted according to the procedure described below.

Adjusting gate “CD4”

1. Click inside gate “CD4” to select it.
2. The frame of the gate changes into a dashed line.
3. Press the  [Confirm] button on the right side of the screen.
4. The histogram switches into full screen mode.
5. Set the left border of the gate.
6. Set the right border of the gate.
7. The gate is confirmed automatically after the second line has been set.

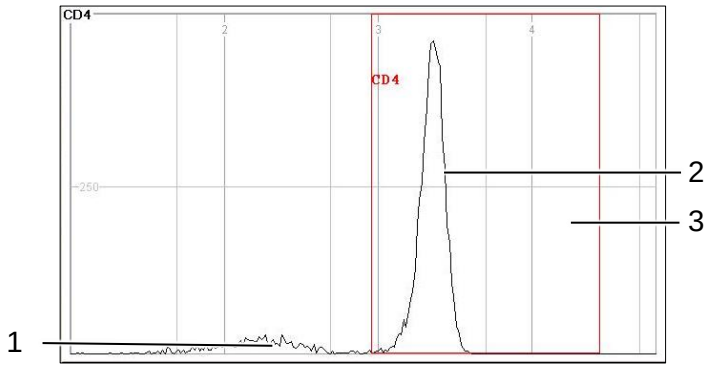


Figure 29: Finished CD4 measurement (blood from a healthy donor)

1	Monocyte peak	3	Gate “CD4”
2	$CD4^+$ T cell peak		

Printout

CD4 measurement results can be printed out:

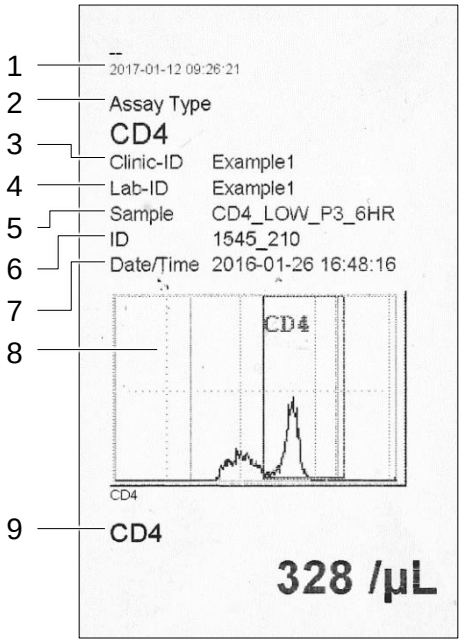


Figure 30: CD4 printout (exemplary)

1	Timestamp of printout (YYYY-MM-DD hour:minute:second)	6	Measurement ID (automatically generated)
2	Assay type (CD4 configuration)	7	Measurement date (YYYY-MM-DD hour:minute:second)
3	Clinic-ID (generated at commissioning)	8	Diagram with caption (CD4)
4	Lab-ID (generated at commissioning)	9	Counting result (cells/µL)
5	Sample ID (user-defined)		

6.6.3 CD4 measurement rating

The following examples are outlining differences and similarities between diagrams to support your understanding of clear and unclear separation. Clear separation is the ideally obvious visible separation between monocytes and $CD4^+$ T lymphocytes, model samples bear a valley as line of separation.

Clear separation

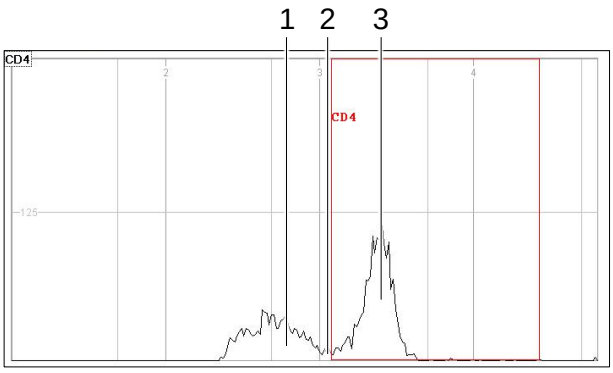


Figure 31: CD4 clear separation (A)

CD4 clear separation (A)

1. Monocytes
2. Valley
3. $CD4^+$ T lymphocytes

High count (high $CD4^+$ T lymphocytes value)

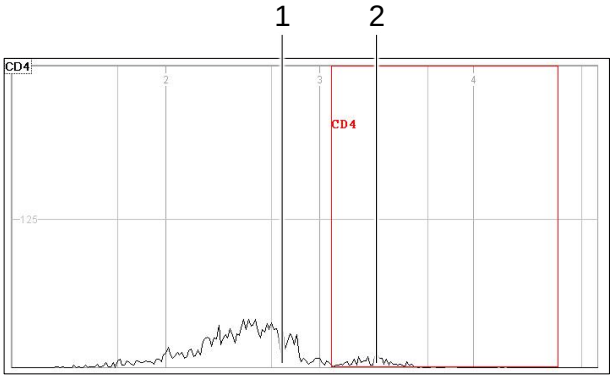


Figure 32: CD4 clear separation (B)

CD4 clear separation (B)

1. Monocytes
2. $CD4^+$ T lymphocytes

Low count ($CD4^+$ T lymphocytes low)

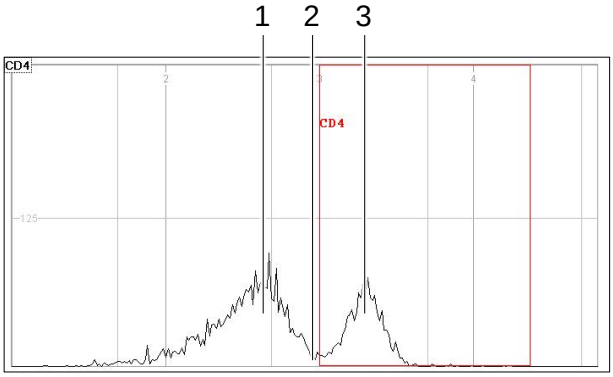


Figure 33: CD4 clear separation (C)

CD4 clear separation (C)

1. Monocytes
2. Valley
3. $CD4^+$ T lymphocytes

Medium count ($CD4^+$ T lymphocytes medium)

Unclear separation

INFORMATION

For information on how to resolve unclear separation, please see chapter “9 Troubleshooting”.

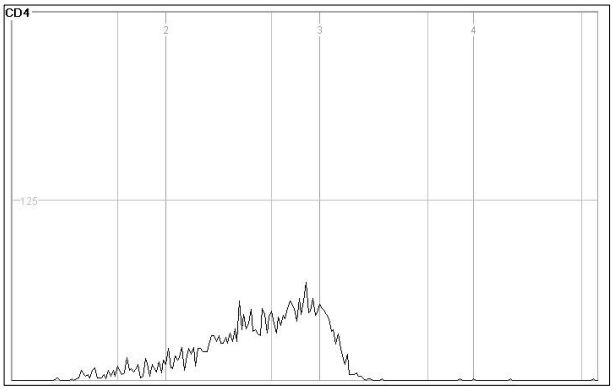


Figure 34: CD4 unclear separation (D)

CD4 unclear separation (D)

No valley

Identification of signals is unclear

No reasonable gating can be applied

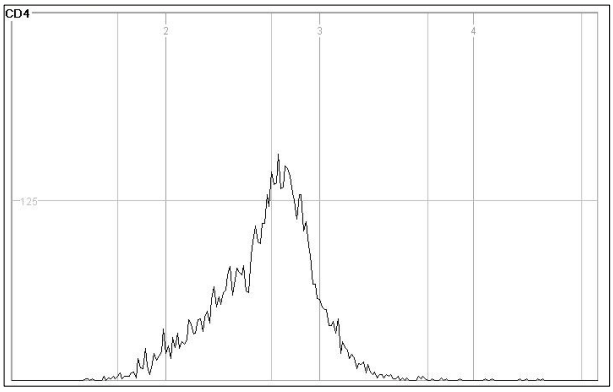


Figure 35: CD4 unclear separation (E)

CD4 unclear separation (E)

No valley

Identification of signals is unclear

No reasonable gating can be applied

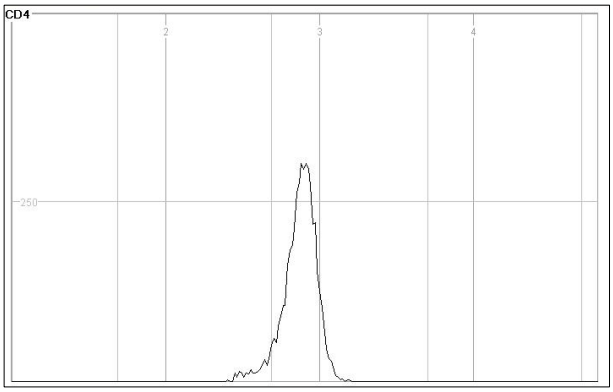


Figure 36: CD4 unclear separation (F)

CD4 unclear separation (F)

No valley

Identification of signals is unclear

No reasonable gating can be applied

6.6.4 CD4% measurement

WARNING

Risk of infection

All human-sourced materials and any objects coming into contact with those materials have the potential to transmit infections.

- Always wear appropriate personal protective equipment.
- Clean and disinfect spills immediately.
- Follow all safety regulations applicable to your institution.

NOTICE

Clotted blood samples

Clotted whole blood samples may compromise measurement results and cause clogging of the fluidic system.

- Do not use clotted blood samples.

To understand the software interface and to use the software correctly, please also see chapter “7 Software”.

Required material

Item	Quantity
EDTA whole blood	1 tube
CD4% easy count kit - CD4 mAb PE - CD45 mAb PE-Cy5 - Buffer 1 - Buffer 2	1 kit
Sample tube 3.5 mL	1 piece
Pipette tips 50-1000 µL	2 pieces
Pipette tips 2-200 µL	3 pieces
Pipette variable 100-1000 µL	1 piece
Pipette fix 20 µL	1 piece

Item	Quantity
Pipette fix 10 µL	1 piece

INFORMATION
After addition of Buffer 1, samples can be stored for up to 2 hours at 2-8 °C in the dark. Do not use samples that have exceeded storage time or that have not been prepared according to the instructions. See also the instructions for use of the CD4% easy count kit.

INFORMATION
After addition of Buffer 2, samples must be analyzed within 10 minutes. It is not recommended to use the sample later than that. See also the instructions for use of the CD4% easy count kit.

CD4% sample preparation

- Invert the blood sample in the EDTA blood collection tube gently 8 to 10 times.
- Pipette 20 µL EDTA whole blood sample to the bottom of an unused sample tube. Avoid leaving a trail of blood from the pipette tip on the inner tube wall. Discard the pipette tip.
- Add 10 µL CD4 mAb PE directly into the blood sample and mix it gently. Avoid leaving a trail of blood from the pipette tip on the inner tube wall. Discard the pipette tip.
- Add 10 µL CD45 mAb PE-Cy5 directly into the blood sample and mix it gently. Avoid leaving a trail of blood from the pipette tip on the inner tube wall. Discard the pipette tip.
- Incubate the mixture for a minimum of 15 minutes and a maximum of 30 minutes at 15 – 30 °C in the dark.
- Add 400 µL Buffer 1 and vortex briefly, or tap tube gently in order to mix the sample. Discard the pipette tip.
- Add 400 µL Buffer 2 and vortex briefly, or mix the sample by pipetting up and down. Discard the pipette tip.

Vortex briefly, or mix the sample by pipetting up and down before analysing with a Sysmex Partec IVD flow cytometer, such as the CyFlow™ Counter.

Measurement

1. Select *Settings* > *Load Configuration* > *CD4percent.scr* from the drop-down menu.
2. Plug the sample tube with the prepared sample gently into the sample port.
3. Press the [*Start*] button.
4. Enter a sample ID (max. 50 characters) via the on-screen keyboard. Confirm by pressing the [*Enter*] button.
5. The ongoing step is displayed in the progress status bar.

The instrument performs a series of steps in the following sequence:

a) Prerun: Creates a temporary, fast sample flow.
b) Measure: The laminar flow is established until TVAC starts. Particles are detected.
c) Level Counting: Particles in a certain volume are detected and counted.
d) Flush: An internal cleaning cycle is performed.

6. Wait until message "*Cleaning …done*" and "*Setting Inject pump…Ready*" is displayed in the Status bars.
   The sample status indicator shows the "Error" symbol.

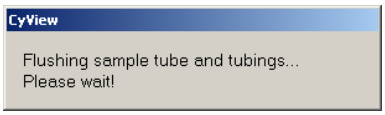


Figure 37: Flushing before CD4% measurement is done

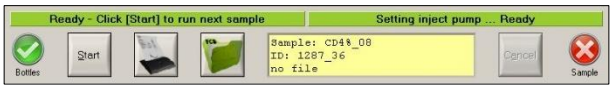


Figure 38: CD4% measurement is done

Measurement diagram

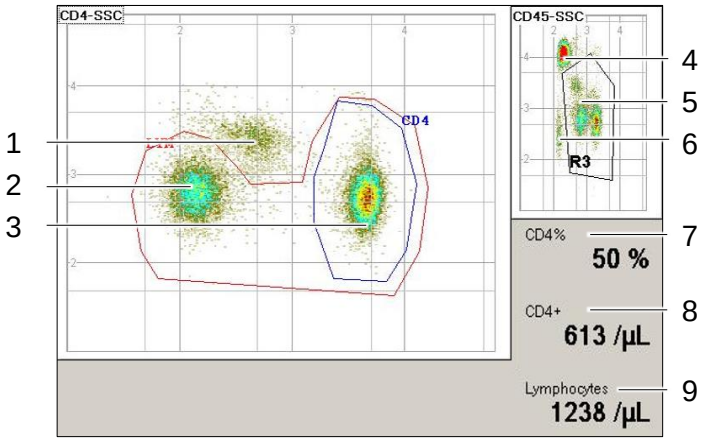


Figure 39: Results of CD4% measurement (example)

1	Monocytes cluster	6	Debris cluster
2	$CD4^-$ lymphocyte cluster, gated by “LYM” gate	7	CD4% result (%)
3	$CD4^+$ T cell cluster, gated by “CD4” gate	8	$CD4^+$ result (cells/µL)
4	Granulocyte cluster	9	Lymphocytes result (cells/µL)
5	Lymphocyte and monocyte clusters, gated by “R3” gate		

Cancelling a measurement

Cancel a measurement by pressing the [*Cancel*] button, while running a measurement.

The following dialogue gives you two options, to either

- Press [*Yes*] and flush the sample tube automatically now.
- Press [*No*] and flush the sample tube manually at a later time.

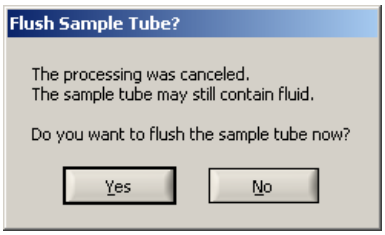


Figure 40: Flush Sample Tube

6.6.5 CD4% measurement post-treatment

Before saving or printing measurement results, verify correct placement of cell populations and debris, if applicable, within the measurement diagram by visual inspection:

- Only lymphocytes and monocytes must be inside the “R3” gate.
- Granulocytes and if present debris must be outside the “R3” gate.
- Only CD4+ T cell must be inside the “CD4+” gate.
- Only CD4+ T cells and CD4- lymphocytes must be inside the “LYM” gate.
- Monocytes must be outside “CD4+” and “LYM” gates.

If these requirements are not met, proceed to adjusting the gates according to the procedure described below. Make sure gate “R3” is adjusted before “CD4+” or “LYM” gates.

INFORMATION

Presence of debris in the measurement diagram is common and is considered a prerequisite to ensure correct adjustment of the “R3” gate.

If no debris population is visible in many samples, please contact your local Sysmex representative.

Adjusting “R3” gate

1. Click inside gate “R3” to select it.
2. The frame of the gate changes into a dashed line.
3. Press the  [Confirm] button on the right side of the screen.
4. The dot plot switches into full screen mode.
5. Click for every corner point to draw a polygon. A maximum of 21 points is possible.
6. Press  to confirm the gate.

Adjusting “CD4” gate

1. Click inside “CD4+” gate to select it.
2. The frame of the gate changes into a dashed line.
3. Press the  [Confirm] button on the right side of the screen.
4. The dot plot switches into full screen mode.
5. Click for every corner point, to draw a polygon. A maximum of 21 points is possible.
6. Press  to confirm the gate.

Adjusting “LYM” gate

1. Click inside “LYM” gate to select it.
2. The frame of the gate changes into a dashed line.
3. Press the  button on the right side of the screen.
4. The dot plot switches into full screen mode.
5. Click for every corner point, to draw a polygon. A maximum of 21 points is possible.
6. Press  to confirm the gate.

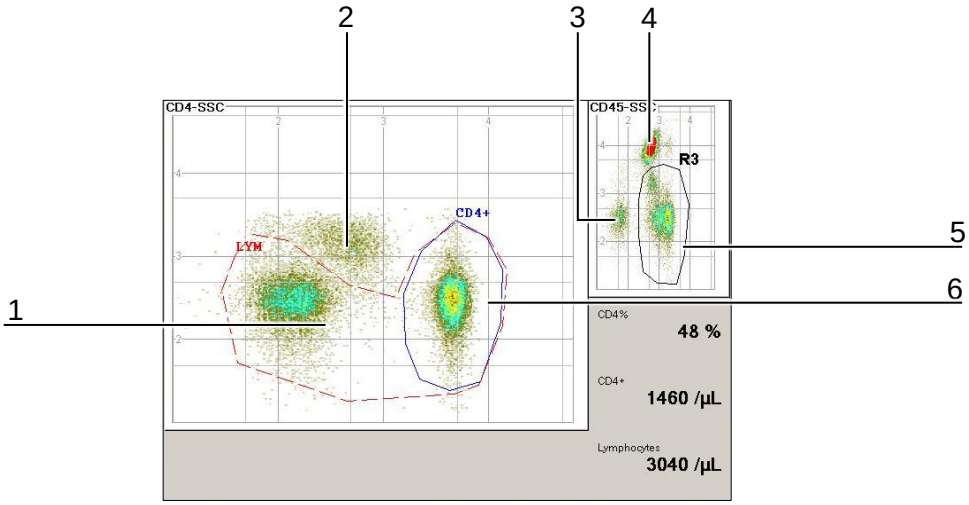


Figure 41: Finished CD4% measurement (blood from a healthy donor)

1	$CD4^+$ T cells and $CD4^-$ lymphocytes, gated by gate “LYM”	4	Granulocytes
2	Monocytes	5	Lymphocytes and monocytes, gated by gate “R3”
3	Debris cluster	6	$CD4^+$ T cells, gated by gate “CD4+” and gate “LYM”

Printout

CD4% measurement results can be printed out.

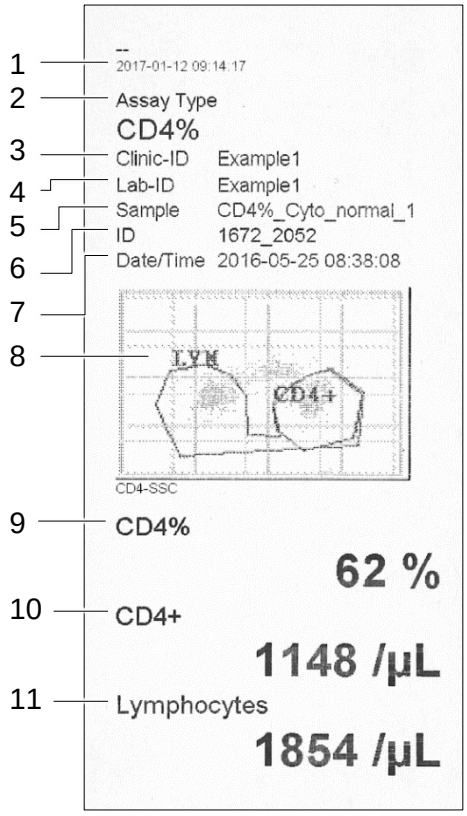


Figure 42: CD4% printout (exemplary)

1	Timestamp of printout (YYYY-MM-DD hour:minute:second)	7	Measurement date (YYYY-MM-DD hour:minute:second)
2	Assay Type (CD4% configuration)	8	Diagram with caption (CD4-SSC)
3	Clinic-ID (generated at commissioning)	9	CD4% result (%)
4	Lab-ID (generated at commissioning)	10	$CD4^+$ result (cells/µL)
5	Sample ID (user-defined)	11	Lymphocytes result (cells/µl)
6	Measurement ID (automatically generated)		

6.6.6 CD4% measurement rating

The following examples are outlining differences and similarities between diagrams to support your understanding of clear and unclear separation. Clear separation is the visible separation between:

For CD4-SSC dot plots: $CD4^-$ lymphocytes and monocytes

For CD45-SSC dot plots: debris cluster and lymphocytes, granulocytes and monocytes

Clear separation

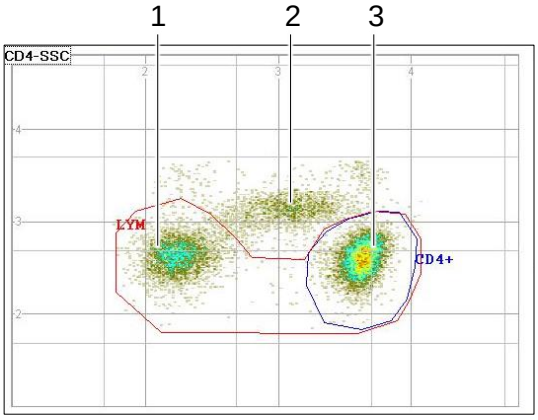


Figure 43: CD4-SSC clear separation (A)

1. $CD4^-$ lymphocytes (“LYM” gate)
2. Monocytes
3. $CD4^+$ T lymphocytes (“LYM” gate and “$CD4^+$” gate)

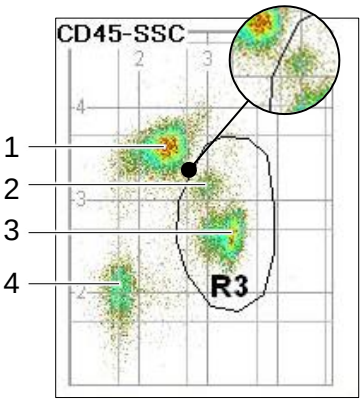


Figure 44: CD45-SSC clear separation (A)

1. Granulocytes
2. Monocytes
3. Lymphocytes
4. Debris

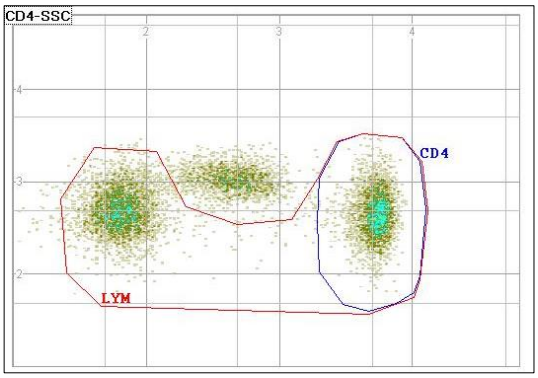


Figure 45: CD4-SSC clear separation (B)

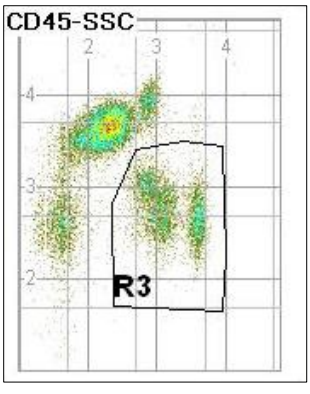


Figure 46: CD45-SSC clear separation (B)

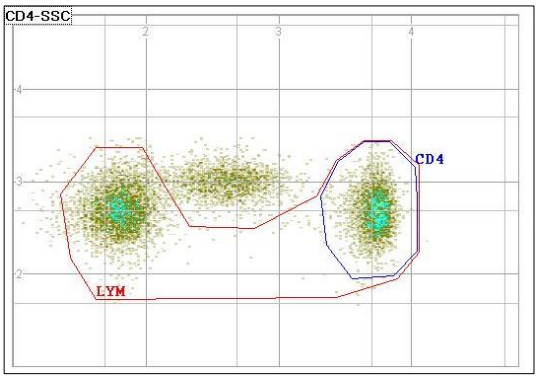


Figure 47: CD4-SSC clear separation (C)

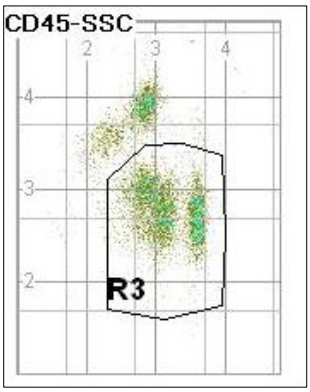


Figure 48: CD45-SSC clear separation (C)

Unclear separation

INFORMATION

For information on how to resolve unclear separation, please see chapter 9 Troubleshooting.

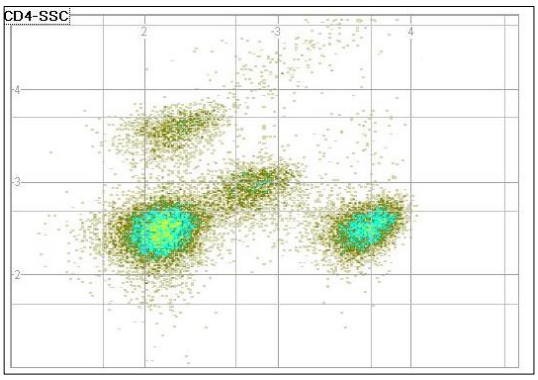


Figure 49: CD4-SSC clear separation (D)

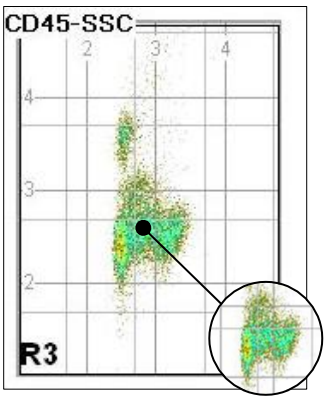


Figure 50: CD45-SSC unclear separation (D)

CD4-SSC identification of signals is clear but CD45-SSC identification of signals is unclear. Granulocytes, monocytes and $CD45^+$ lymphocytes are too close. No reasonable R3 gating can be applied to CD45-SSC.

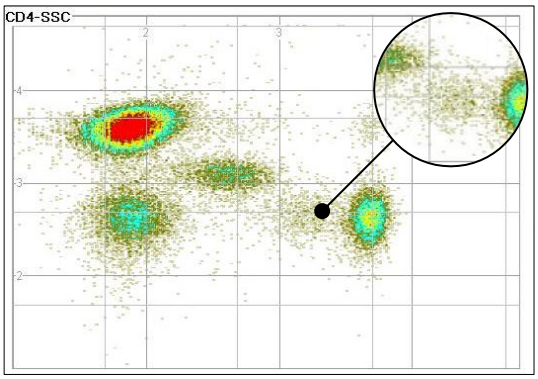


Figure 51: CD4-SSC scattered CD4⁺ cluster (E)

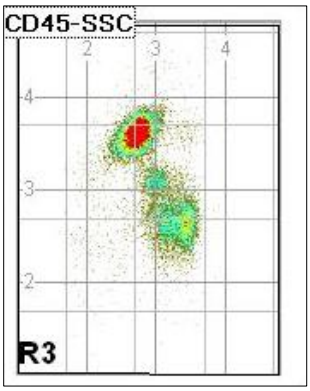


Figure 52: *CD45-SSC clear separation* (E)

Possible indications of insufficient sample preparation: CD4-SSC shows scattered CD4⁺ T lymphocytes. The sample may not have been mixed well.

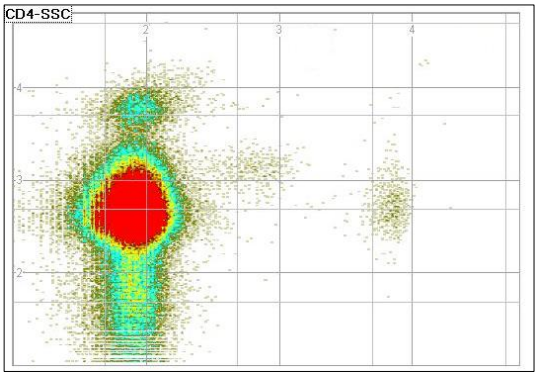


Figure 53: CD4-SSC unclear separation (F)

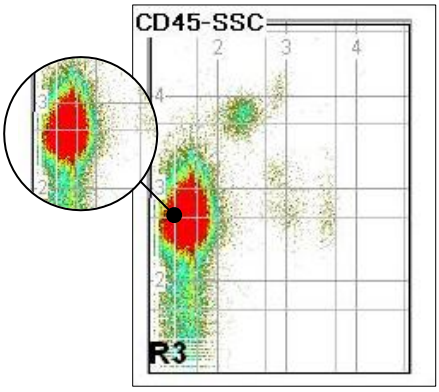


Figure 54: CD45-SSC unclear separation (F)

Irregular signal-to-noise ratio: The cluster suggests a poor signal-to-noise ratio.

6.7 Shutdown

6.7.1 Cleaning

Required material

Item	Quantity
Decontamination Solution	1.6 mL
Cleaning Solution	1.6 mL
Sheath Fluid	1.6 mL
Sample tube 3.5 mL	3 pieces
Pipette tips 50-1000 µL	3 pieces
Pipette variable 100-1000 µL	1 piece

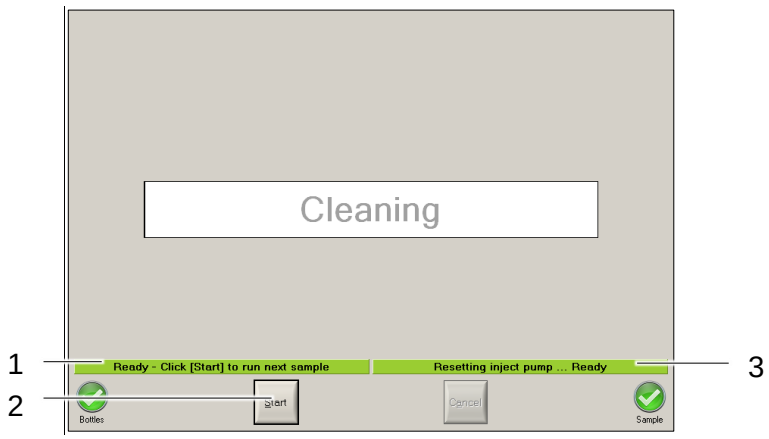


Figure 55: Cleaning

1	Device status bar	3	Progress status bar
2	[*Start*] button		

Procedure

1. Select *Settings* > *Load Configuration* > *Cleaning.scr* from the drop-down menu.
2. Plug a sample tube with 1.6 mL Decontamintation Solution gently into the sample port.
3. Press the [*Start*] button.
4. Wait until message "*Cleaning …done*" and "*Setting Inject pump…Ready*" is displayed in the status bars.
   The sample status indicator shows the "Error" symbol.
5. Plug a sample tube with 1.6 mL Cleaning Solution gently into the sample port.
6. Press the [*Start*] button.
7. Wait until message "*Cleaning …done*" and "*Setting Inject pump…Ready*" is displayed in the status bars.
   The sample status indicator shows the "Error" symbol.
8. Plug a sample tube with 1.6 mL Sheath Fluid gently into the sample port.
9. Press the [*Start*] button.
10. Wait until message "*Cleaning …done*" and "*Setting Inject pump…Ready*" is displayed in the status bars.
    The sample status indicator shows the "Error" symbol.

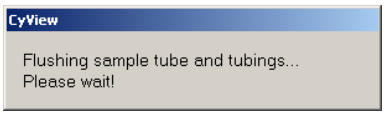


Figure 56: Flushing before Cleaning is done

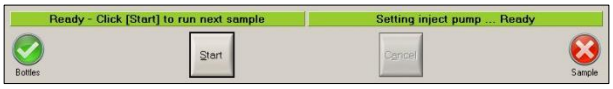


Figure 57: Cleaning is done

6.7.2 Shutting down

NOTICE

Sample port electrodes

The sample port electrodes are most sensitive and are easily damageable. Damaged electrodes can affect detection of the measurement volume and thus lead to incorrect measurement results.

To protect the electrodes when the device is shut down:

- Plug a sample tube filled with 1.6 mL Decontamination Solution gently into the sample port to secure the electrodes.

Press the ON/OFF button once at the device front to initiate a shutdown. Wait for five more seconds after the display went black and no more sounds are emitted. The device is now shut down.

INFORMATION

For devices provided with less than 200 V, the ON/OFF button has to be pressed twice to shutdown the device.

6.7.3 Power off

NOTICE

White screen

For devices provided with less than 200 V, when the device is shut down, the display shows a white screen. If a white screen is shown, internal electronics may get damaged if the device is not powered off.

- Power the device off at the end of each working day by pressing the main power switch on the rear side of the instrument.
- Only restart a device, if it was powered off on the rear side of the instrument.

Only power off the device if it is shut down. Press the main power switch at the back of the device to power off the device.

6.7.4 Long-term shutdown and storage

In case the device will be idle for a time between two and six days, make sure to shut the device down and cover it with the protective cover. Weekly cleaning is required latest after 7 days.

It is not recommended to keep the system idle for longer than seven days. Keeping the system idle for longer than seven days without weekly cleaning may result in Sheath Fluid crystallization or other residual particles in the tubing and a compromised fluidic system.

Please contact your local Sysmex representative if you need to shutdown and store your device for a longer period of time.

7 Software

7.1 Overview

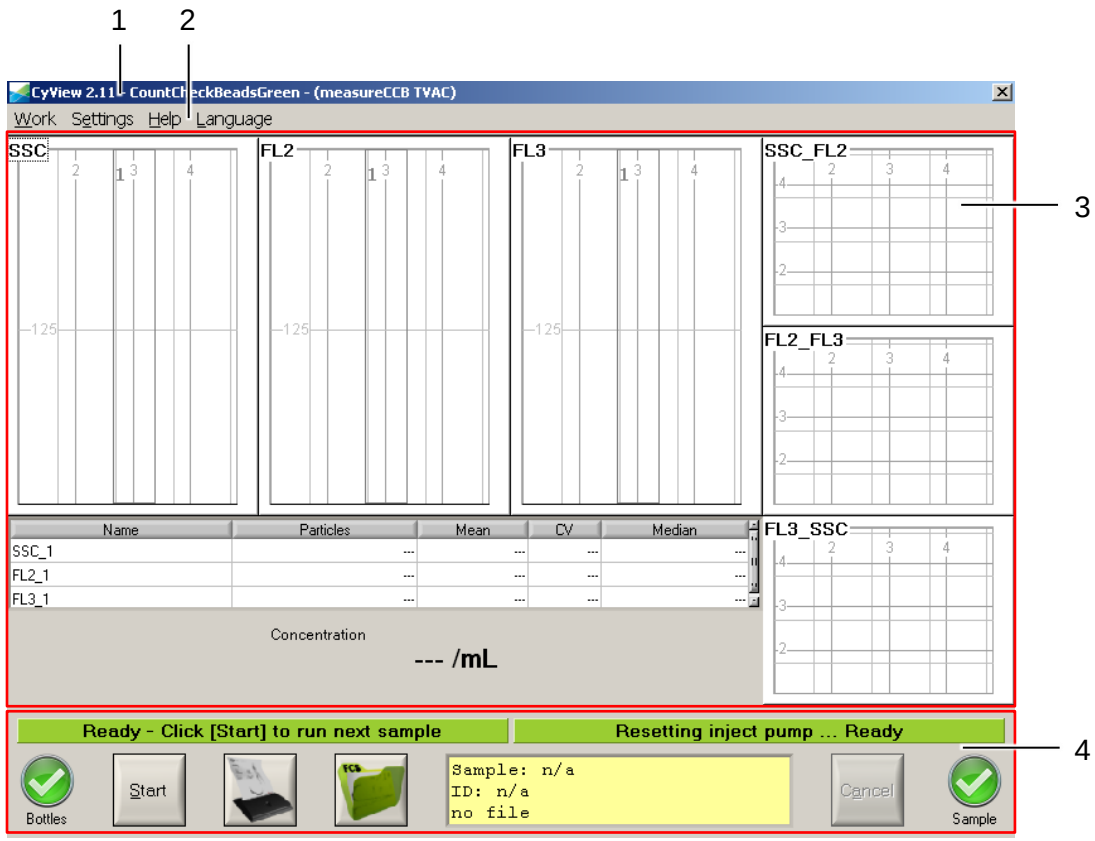


Figure 58: User interface screen (example)

1	Title bar	3	Main area with diagrams
2	Menu bar	4	Bottom menu

7.1.1 Title bar

The title bar contains the following information:

Figure 59: Title bar (example)

Software name and version number:	*CyView 2.11*
Active function:	*Cleaning*
Active configuration:	*(measureClean)*

7.1.2 Menu bar

Figure 60: Menu bar

Table 9: Menu bar drop-down structure

Configuration	Work	Settings	Help	Language
Cleaning	*Reload Data*	*Load Configuration*	*Program Help*	*English*
			About	*German*
CD4	*Reload Data*	*Load Configuration*	*Program Help*	*English*
		Save Configuration	*About*	*German*
		Data Directory Settings		
CD4%	*Reload Data*	*Load Configuration*	*Program Help*	*English*
		Save Configuration	*About*	*German*
		Data Directory Settings		
Count Check Beads green	*Reload Data*	*Load Configuration*	*Program Help*	*English*
		Data Directory Settings	*About*	*German*
Emergency Cleaning	*Reload Data*	*Load Configuration*	*Program Help*	*English*
			About	*German*

Work

- *Reload Data*: Reload a saved data file including the layout (gates & scaling). It is possible to set gates anew and to save the data file again.

INFORMATION

If a reloaded data file is modified and saved again, the file name for the new file is the original name plus the extension "_mod". A second modification will not occur (there will not be an extension "_mod_mod").

- The modified file name is: date_time_file name_mod.fcs)
- Data files are saved as ".fcs" files (Flow cytometry standard)

Settings

- *Load Configuration*: A configuration is a file containing the layout (gates and scaling) as well as default software parameters (Gain and Lower-Level). The configuration enables the associated function.
- The following configurations are loadable:
  - CD4 (CD4.scr)
  - CD4% (CD4percent.scr)
  - Cleaning (Cleaning.scr)
  - Count Check Beads green (CountCheckBeadsGreen.scr)
  - Emergency Cleaning (EmergencyCleaning.scr)
- *Save Configuration*: Secures changes to a configuration.
- *Data Directory Settings…*: Create an additional sub-directory automatically "Per Day", "Per Week" or "Per month". "None" is default.

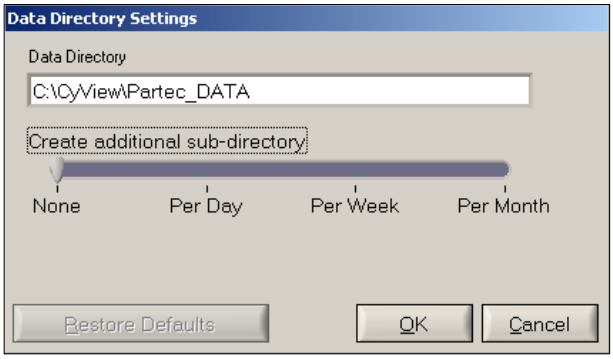


Figure 61: Data Directory Settings

Help

- *Program Help*: Opens the program help. The Instructions for Use of the device are displayed as ".pdf" file.
- *About*: Shows information about software and firmware identification.

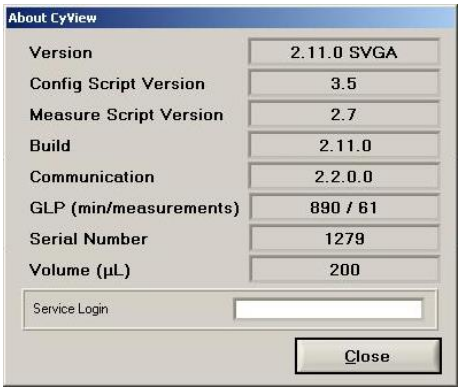


Figure 62: About CyView (exemplary)

Language

Choose one of these languages in this drop-down menu: *English* (default), *German*. This will change the interface language of the software.

7.1.3 Main area diagrams

CD4 histogram

The gate of a CD4 histogram is adjustable.

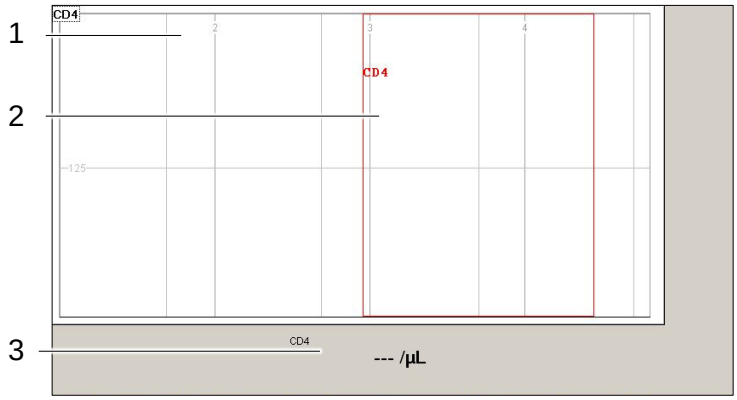


Figure 63: CD4 histogram (before measurement)

1	Histogram (CD4)	3	CD4 measurement result (cells/µL)
2	Gate “CD4”		

CD4% dot plot

Gates of a CD4% dot plot are adjustable.

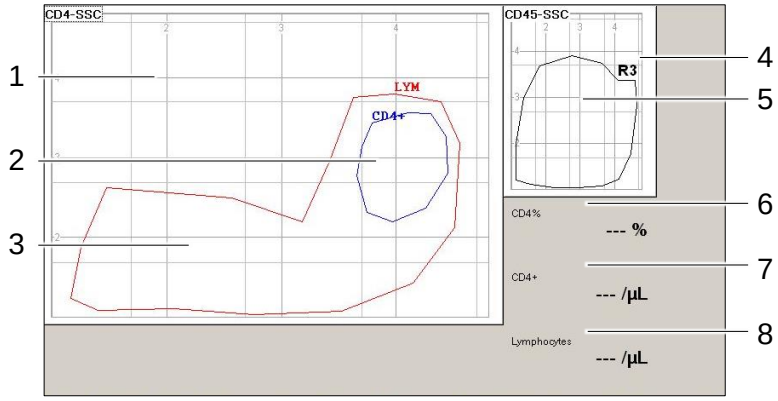


Figure 64: CD4% dot plot (before measurement)

1	Dot plot (CD4-SSC)	5	Gate “R3”
2	Gate “CD4+”	6	CD4% result (%)
3	Gate “LYM”	7	CD4 result (cells/µL)
4	Dot plot (CD45-SSC)	8	Lymphocytes result (cells/µL)

CCBg histograms and dot plots

Gates of CCBg histograms and dot plots are not adjustable.

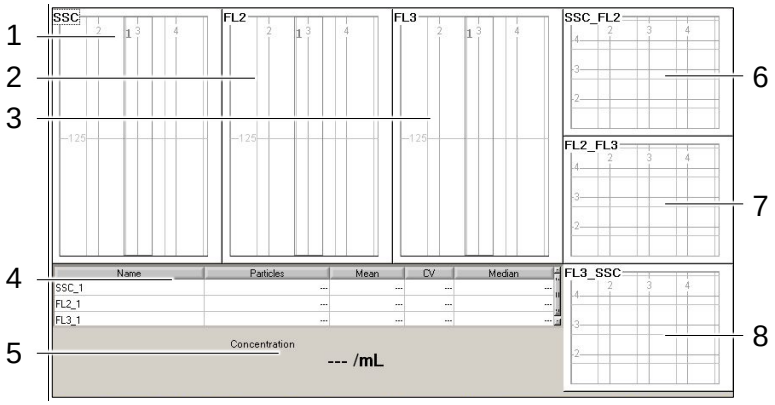


Figure 65: CCBg histograms and dot plots (before measurement)

1	Histogram (SSC)	5	Concentration (CCBg/mL)
2	Histogram (FL2)	6	Dot plot (SSC_FL2)
3	Histogram (FL3)	7	Dot plot (FL2_FL3)
4	Measurement results for SSC, FL2 and FL3	8	Dot plot (FL3_SSC)

7.1.4 Bottom menu

Bottom menu layout varies depending on the active configuration.

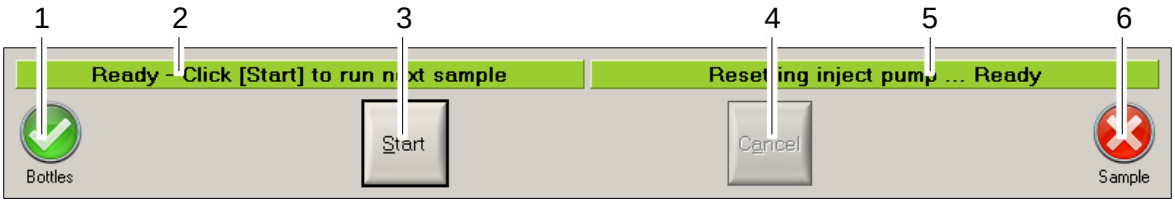


Figure 66: Cleaning configuration (before measurement)

1	Bottle status indicator	4	[*Cancel*] button
2	Device status bar	5	Progress status bar
3	[*Start*] button	6	Sample status indicator

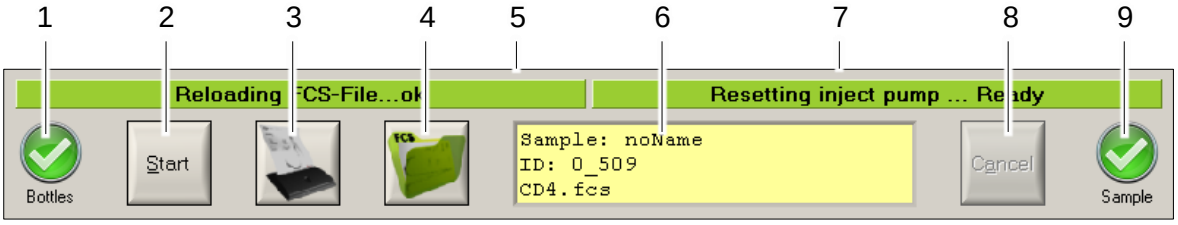


Figure 67: CD4, CD4% and Count Check Beads green configurations

1	Bottle status indicator	6	Sample identification
2	[*Start*] button	7	Progress status bar
3	[Print] button	8	[*Cancel*] button
4	[Save] button	9	Sample status indicator
5	Device status bar		

Both bottle status indicator and sample status indicator can show the “Ready” or “Error” symbol.

The “Ready” symbol at the bottle status indicator points out, that the contents of either bottles are ready for proceeding. The “Ready” symbol at the sample status indicator points out, that the content of the sample tube is ready for proceeding.

The “Error” symbol indicates always an error, e.g. a Sheath bottle connection is lost, Sheath bottle is empty, the waste bottle is full or the sample tube is empty and so on.

Additionally the bottle status indicator can show an “Attention” symbol. It indicates a filling status error of either bottle only during measurements, meaning the Sheath bottle is empty or the waste bottle is full.

Figure 68: "Ready" symbol

Figure 69: "Error" symbol

Figure 70: "Attention" symbol

Save

The acquired data, layout and software parameters can be saved by pressing the [Save] button.

The default name for a file is: *date_time_name.fcs*

INFORMATION
If a reloaded data file is modified and saved again, the file name for the new file is the original name plus the extension “_mod”. A second modification will not occur (there will not be an extension “_mod_mod”). - The modified file name is: date_time_file name_mod.fcs) - Data files are saved as “.fcs” files (Flow cytometry standard)

Print

The numerical results along with the histograms and/or dot plots can be printed by pressing the [Print] button.

INFORMATION
The printer utilizes thermal paper. Thermal paper is not suited for long-term storage. For permanent or longterm storage make a copy on regular paper.

7.2 On-screen keyboard

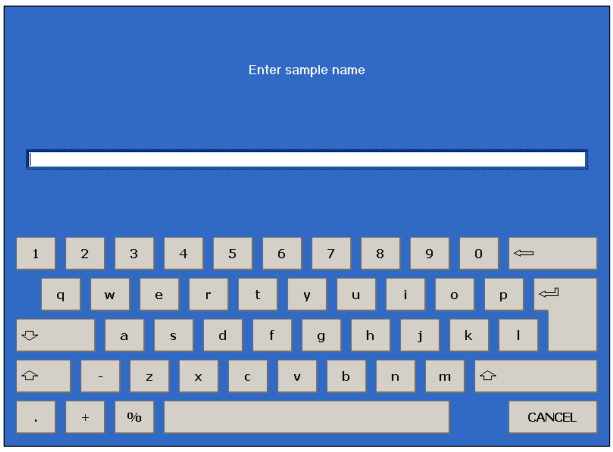


Figure 71: On-screen keyboard (before measurement)

If data input is necessary (e.g. starting a measurement), an on-screen keyboard opens automatically on the screen.

Confirm input by pressing the [*Enter*] button. After confirmation, the software keyboard closes automatically.

7.3 Gates

Each configuration has one or more predefined gates to analyse a sample.

A gate is used to selectively visualize cells of interest while eliminating results from unwanted particles (e.g. debris). Gates can be adjusted but never deleted.

The gate type in a histogram is called range. Range is the space between the two borders.

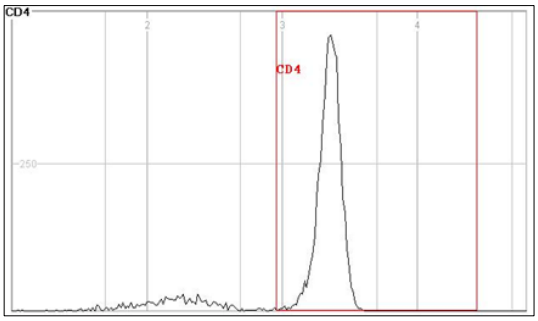


Figure 72: Histogram (example)

The gate type in a dot plot is called polygon. A polygon can have between 3 and 21 points for its border.

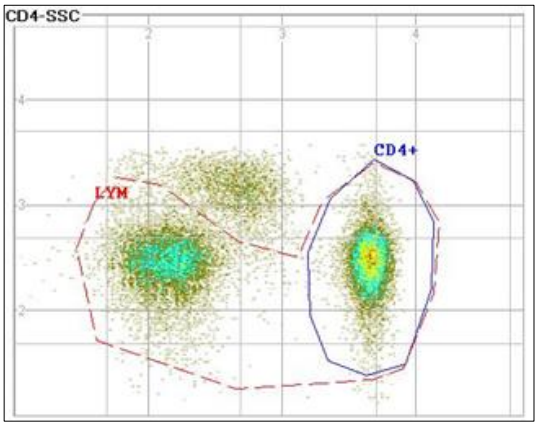


Figure 73: Dot plot (example)

Table 10: Configurations, gates and gated signals

Configuration	Gate type	Gate name	Gated signals
CountCheckBeadGreen.scr	Range	SSC_1	CCBg
	Range	FL2_1	CCBg
	Range	FL3_1	CCBg
CD4.scr	Range	CD4	$CD4^+$ T cell
CD4percent.scr	Polygon	R3	Lymphocytes and monocytes
	Polygon	CD4+	$CD4^+$ T cell
	Polygon	LYM	$CD4^+$ T cell
			$CD4^-$ lymphocytes

INFORMATION

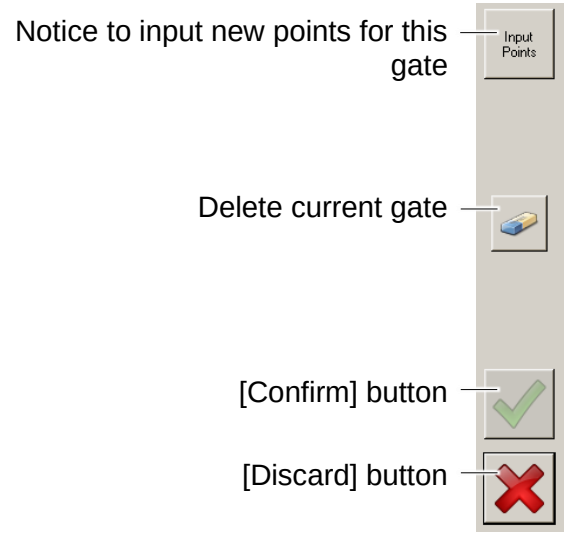
The configuration “CD4percent.scr” has an active “R3” gate.

That means only displayed signals which are inside the “R3” gate will be displayed in the “CD4-SSC” dot plot and the “CD45” histogram.

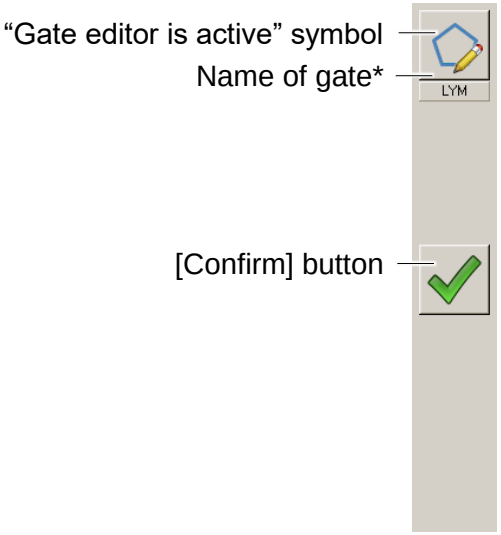
Gate editing

Click into the respective gate to change the line of the gate to dashes. On the right side of the diagram click the  [Confirm] button to initiate the Gate editor.

Gate editor



Gate editor confirmation



*Gate names for CD4 measurement: “CD4”

*Gate names for CD4% measurement: “LYM”, “CD4+”, “R3”

8 Maintenance

This section describes maintenance activities that you need to perform at specified intervals to ensure proper instrument function.

In case of questions about the device or any service needs, or if the device fails to operate properly, please contact your local Sysmex representative. Do not attempt to repair the device yourself.

8.1 Frequency of maintenance tasks

Table 11: Frequency of maintenance tasks

Activity	Daily	Weekly	Every 3 months	Every 12 months	As-needed
Start-up cleaning	x				
Shutdown cleaning	x				
Shutdown	x				
Empty liquid waste	x				
Empty and refill Sheath	x				
Weekly cleaning		x			
Cleaning device exterior		x			
Inline filter replacement			x		
Printer paper refilling					x
Emergency cleaning					x
Preventive maintenance				x	

8.2 Daily maintenance

8.2.1 Start-up cleaning

Start-up cleaning is part of daily start-up of the device. Please refer to section 6.3.2 Cleaning for detailed information.

8.2.2 Shutdown cleaning and shutdown

Shutdown cleaning is performed as part of the shutdown process. Please refer to section 6.7.1 Cleaning for detailed information.

In case the device will be idle for a time between two and six days, make sure to shut the device down and cover it with the protective cover. Weekly cleaning is required latest after 7 days.

Please contact your local Sysmex representative if you need to shutdown and store your device for a longer period of time.

8.2.3 Empty liquid waste

Perform this procedure at the end of each working day. Make sure the device is switched off and no vacuum is applied to the device and its components.

Before emptying the waste bottle, the content of the bottle needs to be treated with 0.5 percent hypochlorite solution to reduce the protein content in the waste fluid. Use an amount of hypochlorite solution of about 10% of the waste bottle content (example: 800 mL content = 80 mL hypochlorite solution) and follow the steps below.

Required material

Item	Quantity
Hypochlorite solution (0.5%)	10% of waste bottle content
Disinfective agent (alcohol-free, free of organic solvents)	as needed
Clean cloth	1 piece

Prerequisite

- Device is switched off
- Wear appropriate personal protective equipment

WARNING

All human-sourced materials and any objects coming into contact with those materials have the potential to transmit infections.

- Always wear appropriate personal protective equipment.
- Dispose of all materials considered potentially infectious in accordance with local regulations.

INFORMATION

When screwing or unscrewing a bottle and its respective cap, it is advantageous to twist the bottle. The tubing must stay unbent.

Procedure

1. Unscrew the waste bottle.
2. Secure cap and tubing on a clean surface in a stable manner.
3. Add a sufficient amount of Hypochlorite solution (10% of waste volume) to the waste bottle.
4. Let bottle contents incubate for 15 minutes.
5. Dispose of liquid waste in accordance with local regulations.
6. Screw down the cap by turning the bottle. Do not twist the tubing and the sensor cable. Close the bottle tightly.
7. Wipe bottle exterior and working surface with a clean cloth moistened with disinfective agent.
8. Wipe bottle with clean cloth.
9. Dispose of cloth.

8.2.4 Empty and refill Sheath Fluid

Perform this procedure at the beginning of a new working day directly before starting to use the device.

INFORMATION
When screwing or unscrewing a bottle and its respective cap, it is advantageous to twist the bottle. The tubing must stay unbent.

Required material

Item	Quantity
Sheath Fluid	max. 900 mL
Clean cloth	1 piece

Prerequisite

- Wear appropriate personal protective equipment

Procedure

1. Unscrew the Sheath bottle.
2. Pull the tubing including the inline filter out of the Sheath bottle.
3. Put cap and tubing including the inline filter on a clean surface in a stable manner.
4. Dispose of Sheath Fluid in accordance with local regulations.
5. Wipe bottle with clean cloth.
6. Dispose of cloth.
7. Fill the bottle with up to 900 mL of Sheath Fluid.
8. Insert the tubing including the inline filter into the bottle.
9. Move the cap gently up and down several times, while keeping the inline filter in Sheath Fluid to remove air bubbles from the inline filter.
10. Screw down the cap by turning the bottle. Do not twist the tubing and/or the sensor cable.

8.3 Weekly maintenance

8.3.1 Weekly cleaning

Perform this cleaning procedure once a week to clean the device's sample pathways and to prevent build-up of proteins and contaminants in the system and fluidics bottles.

Required material

Item	Quantity
Cleaning Solution	1.6 mL
Decontamination Solution	1.6 mL
Sheath Fluid	1.6 mL
Sample tube 3.5 mL	3 pieces
Pipette tips 50-1000 µL	3 pieces
Pipette variable 100-1000 µL	1 piece

Prerequisite

- Wear appropriate personal protective equipment

Procedure

1. Select *Settings* > *Load Configuration* > *EmergencyCleaning.scr* from the drop-down menu.
2. Plug a sample tube with 1.6 mL Decontamination Solution gently into the sample port.
3. Press the [*Start*] button.
4. Wait until message "*Cleaning …done*" and "*Setting Inject pump…Ready*" is displayed in the status bars.
   This step will take 15 minutes to complete. The sample status indicator shows the "Error" symbol.
5. Select *Settings* > *Load Configuration* > *Cleaning.scr* from the drop-down menu.
6. Plug a sample tube with 1.6 mL Cleaning Solution gently into the sample port.
7. Press the [*Start*] button.

8. Wait until message "*Cleaning …done*" and "*Setting Inject pump…Ready*" is displayed in the status bars.
   The sample status indicator shows the "Error" symbol.
9. Plug a sample tube with 1.6 mL Sheath Fluid gently into the sample port.
10. Press the [*Start*] button.
11. Wait until message "*Cleaning …done*" and "*Setting Inject pump…Ready*" is displayed in the status bars.
    The sample status indicator shows the "Error" symbol.

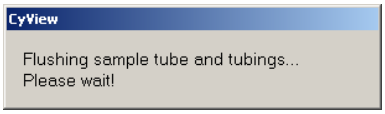


Figure 74: Flushing before Cleaning is done

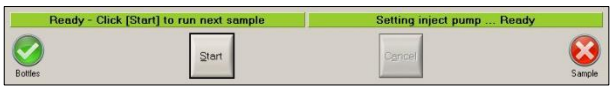


Figure 75: Cleaning is done

8.3.2 Cleaning the device exterior

Perform this procedure at least once a week to ensure smooth operation.

WARNING

All human-sourced materials and any objects coming into contact with those materials have the potential to transmit infections.

- Always wear appropriate personal protective equipment.
- Dispose of all materials considered potentially infectious in accordance with local regulations.

NOTICE

Sample port electrodes

The sample port electrodes are most sensitive and are easily damageable. Damaged electrodes can affect detection of the measurement volume and thus lead to incorrect measurement results.

- Only authorised service personnel is allowed to clean the sample port and its components.
- Do not touch the sample port electrodes.

NOTICE

Harmful substances

Using unsuitable agents for cleaning and disinfection may result in damages to the surfaces of the device and the touchscreen.

- Always use alcohol-free cleaning agents for cleaning device surfaces.
- Do not use abrasive cleaners.

Required material

Item	Quantity
Cleaning agent (alcohol-free, free of organic solvents)	as needed
Disinfective agent (alcohol-free, free of organic solvents)	as needed
Screen cleaner	as needed
Soft, lint-free cloth	4 pieces

Prerequisite

- Device is switched off
- Wear appropriate personal protective equipment

Procedure

To clean the touchscreen:

1. Moisten a fresh lint-free cloth with screen cleaner.
2. Wipe the touchscreen gently until its surface is clean.

To clean the device exterior:

3. Moisten a fresh lint-free cloth with cleaning agent.
4. Thoroughly wipe the exterior of the device as required.

In case of spots and stains of biohazardous substances on the housing or on components:

5. Moisten a fresh lint-free cloth with disinfective agent.
6. Thoroughly wipe the affected area.
7. Wipe the area with a dry clean cloth.

Dispose of cloths after cleaning is done.

8.4 Quarterly maintenance

8.4.1 Inline filter renewal

WARNING

All human-sourced materials and any objects coming into contact with those materials have the potential to transmit infections.

- Always wear appropriate personal protective equipment.
- Dispose of all materials considered potentially infectious in accordance with local regulations.

Required material

Item	Quantity
Inline filter	1 piece

Prerequisite

- Wear appropriate personal protective equipment

Procedure

1. Unscrew the Sheath bottle.
2. Pull the tubing, including the inline filter, out of the Sheath bottle.
3. Put cap and tubing, including the inline filter, on a clean surface in a stable manner.
4. Pull the used inline filter off the tubing (do not pull the tubing off the cap).
5. Dispose of the used inline filter.
6. Attach a new inline filter to the tubing.
7. Insert the tubing, including the inline filter, into the Sheath bottle.
8. Move the cap gently up and down several times while keeping the inline filter covered in Sheath Fluid to remove air bubbles from the inline filter.
9. Screw down the cap by turning the bottle. Do not twist the tubing and the sensor cable.
10. Dispose the used inline filter in accordance with applicable local regulations.

8.5 As-needed maintenance

8.5.1 Emergency cleaning

Perform this cleaning procedure when you are instructed to do so as part of a troubleshooting procedure, e. g. when internal quality control is out of range.

WARNING

Toxic

Acidification of Hypochlorite generates poisonous hypochlorous acid. Contact with hypochlorous acid causes poisoning and irritates body organs.

- Never let Hypochlorite Solution get in contact with acids, like acetic acid or other acidic cleaning agents.
- Always rinse materials treated with Hypochlorite Solution with neutral fluids after use.

NOTICE

Aggressive chemical

Hypochlorite Solution can damage the tubings of the instrument.

- Do not let hypochlorite stay in contact with the tubing for longer than 30 minutes.

Required material

Item	Quantity
Cleaning Solution	1.6 mL
Decontamination Solution	1.6 mL
Sheath Fluid	1.6 mL
Hypochlorite Solution	1.6 mL
Sample tube 3.5 mL	4 pieces
Pipette tips 50-1000 µL	4 pieces
Pipette variable 100-1000 µL	1 piece

Prerequisite

- Wear appropriate personal protective equipment

Procedure

1. Select *Settings* > *Load Configuration* > *EmergencyCleaning.scr* from the drop-down menu.
2. Plug a sample tube with 1.6 mL Hypochlorite Solution gently into the sample port.
3. Press the [*Start*] button.
4. Wait until message "*Cleaning …done*" and "*Setting Inject pump…Ready*" is displayed in the status bars.
   This step will take 15 minutes to complete. The sample status indicator shows the "Error" symbol.
5. Plug a sample tube with 1.6 mL Decontamination Solution gently into the sample port.
6. Press the [*Start*] button.
7. Wait until message "*Cleaning …done*" and "*Setting Inject pump…Ready*" is displayed in the status bars.
   This step will take 15 minutes to complete. The sample status indicator shows the "Error" symbol.
8. Select *Settings* > *Load Configuration* > *Cleaning.scr* from the drop-down menu.
9. Plug a sample tube with 1.6 mL Cleaning Solution gently into the sample port.
10. Press the [*Start*] button.
11. Wait until message "*Cleaning …done*" and "*Setting Inject pump…Ready*" is displayed in the status bars.
    The sample status indicator shows the "Error" symbol.
12. Plug a sample tube with 1.6 mL Sheath Fluid gently into the sample port.
13. Press the [*Start*] button.
14. Wait until message "*Cleaning …done*" and "*Setting Inject pump…Ready*" is displayed in the status bars.
    The sample status indicator shows the "Error" symbol.

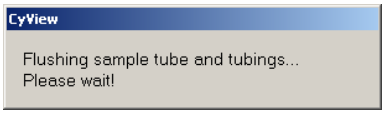


Figure 76: Flushing before Cleaning is done

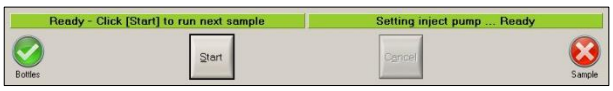


Figure 77: Cleaning is done

8.5.2 Printer paper renewal

INFORMATION
If the paper tray cover is not closed correctly but the [Print] button is pushed, the print job is queued. After closing the cover correctly, all print jobs in the queue will be processed.

Required material

Item	Quantity
Thermal printer paper roll	1 piece

Procedure

1. Open paper tray cover by pulling the latch.
2. Remove the empty paper roll from the paper tray.
3. Unwind about 3 cm of the paper roll and insert the roll into the printer as the picture below suggests.
4. Close the paper tray cover. There should be an audible click. The unrolled part of the paper must stick out when closing the cover.
5. Press the Feed button or the Test print button and check function.
   If the status LED is lit and paper is fed, the paper roll was inserted correctly.
   If the status LED blinks and no paper is fed, readjust the paper roll.

Figure 78: Correctly inserted printer paper

8.6 Preventive maintenance

Preventive maintenance is part of the technical service responsibilities and must only be performed by authorised service personnel of Sysmex. To ensure a secure and adequate functional condition of your device, please contact your local Sysmex representative.

9 Troubleshooting

9.1 Fault, cause and remedy

The information in this section is designed to help you troubleshoot certain issues or conditions that may arise during operation of your device. If the fault you are experiencing is not described or the remedy could not solve your problem, please contact your local Sysmex representative.

If access to CyView™ should be restored, please shut down the device by pushing the ON/OFF button and start it again.

Table 12: Fault, cause and remedy

Fault	Possible cause	Remedy
Internal Quality Check out of the +/-10% range	Inhomogeneous CCBg suspension	Shake CCBg bottle thoroughly for 15 seconds and repeat measurement
		Check if the CCBg are deteriorated
	Air bubbles	Please see chapter 0 Internal Quality Check out of range
		Perform Emergency Cleaning
	Disturbed flow in flow cuvette	Perform Emergency Cleaning
Level counting phase sometimes takes up to 4 min even with high sample speed and sample flow is very slow or is not running	Pressure loss	Make sure the waste bottle is closed properly and the bottle does not leak
	Disturbed flow in flow cuvette	Perform Emergency Cleaning
The measurement is finished in less than 2 minutes even with low sample speed	Sheath Fluid flow too low	Change the inline filter
		Check if tubing is blocked or bent

Fault	Possible cause	Remedy
		Check if external Sheath Fluid tubing is bent or blocked
The measurement is finished in less than 2 minutes even with low sample speed	Insufficient sample preparation	Prepare new sample
	Poor quality of Sheath Fluid (turbidity or visible contamination)	Change Sheath Fluid
	Blocked inline filter	Change inline filter
A very broad and fast rising peak/cluster is visible	Insufficient sample preparation	Prepare new sample
	Poor quality of Sheath Fluid (turbidity or visible contamination)	Change Sheath Fluid
	Blocked inline filter	Change inline filter
Sample run does not start after pressing the [*Start*] button	Sample fluid level not detected	Check the sample tube for the correct volume
No histogram or dot plot visible during the measure phase	Wrong configuration	Load correct configuration
	Insufficient sample preparation	Prepare new sample
	Disturbed flow in flow cuvette	Perform Emergency Cleaning
Unclear separation between the specific peak/cell cluster and the background signals	Abnormal blood quality	Perform Internal Quality Check
	Insufficient sample preparation	Prepare new sample
	Contaminated fluid or components	Perform Internal Quality Check
		Make sure the expiry date of the reagents used is not exceeded.
		Change Sheath Fluid
	Blocked inline filter	Change inline filter

Fault	Possible cause	Remedy
	Poor quality of anticoagulant	Make sure the recommended anticoagulant (EDTA) was used for sample collection
	Blood sample too old	The blood sample should be fresh, i.e. not more than six hours should have elapsed between sample collection and analysis
The device emits loud noise	Waste bottle leaks	Check waste bottle for cracks
		Check if the waste bottle cap is screwed down correctly
	Air tubing leaks	Check external air tubing
Very broad peaks and poor separation between the peaks/cell clusters	Poor sample quality or poor sample preparation	Check sample integrity (blood collection system, storage, preparation procedure)
		Perform Internal Quality Check
	Poor reagent quality	Make sure the expiry date of the reagents used is not exceeded.
	Disturbed flow in flow cuvette	Perform Emergency Cleaning
Instrument off after sudden power cut while sample is being measured	Cable unplugged / Power source cut off	Switch on and immediately start an internal cleaning cycle
Message "*Check bottles*"	Sheath bottle empty and/or waste bottle full	Refill Sheath bottle and/or empty waste bottle after measurement
	Sheath and/or waste bottle caps and connections are loose	Make sure bottle caps are screwed on tightly. Make sure tubing and sensor cable connections are properly installed.
Message "*Inject pump device error*"	Undefined communication status between controller card and syringe pump	Reload configuration

Fault	Possible cause	Remedy
		Restart device
Message "*The available disk space is lower than 600 MB.*"	The remaining disk space is 600 MB or lower. The warning is constantly shown, as long as < 600MB remain.	Upon reaching 200 MB remaining disk space, no further data files can be saved. For data transfer please contact your local Sysmex representative.
Message "*Not enough disk space available to store measurement data files*."	The remaining disk space is 200 MB or lower. No further data files can be stored.	Please contact your local Sysmex representative
Boot error message: "*Windows could not start because the following file is missing or corrupt: \WINDOWS\SYSTEM32\ CONFIG\SYSTEM*"	Corrupt installation	Please contact your local Sysmex representative
True Volumetric Absolute Counting failed	Measured sample volume incorrect	Repeat measurement If this failure occurs three times in a row or more than every 4th measurement, please contact your local Sysmex representative.
Status LED of thermal printer blinks	Thermal printer paper is empty / not inserted	Insert new thermal printer paper
	Thermal printer paper inserted incorrectly	Readjust thermal printer paper
	Thermal printer is not closed correctly	Close the thermal printer
Status LED of thermal printer is static	Printout is empty	Readjust thermal printer paper
		Use other thermal printer paper

9.2 Internal Quality Check out of range

Description

The result of the Count Check Beads green (CCBg) measurement is out of the +/- 10% range of the lot-specific concentration stated on the CCBg bottle label.

Remedy

Proceed according to the following sections depending on the fault details.

If the following procedures do not eliminate the fault, perform emergency cleaning procedure as described in section “8.5.1 Emergency cleaning”.

9.2.1 Narrow and tall peaks visible within the gate

Narrow and tall peak visible within the gate. Check that the displayed CV% values are within the following limits:

SSC ≤ 6%
FL2 ≤ 4%
FL3 ≤ 4%

Remedy

1. Shake the dissolved CCBg bottle thoroughly for 20 seconds.
2. Repeat measurement.

9.2.2 Wide not tall peaks

The peak is wide not tall. Check that the displayed CV% values are within the following limits:

SSC ≤ 6%
FL2 ≤ 4%
FL3 ≤ 4%

Remedy

1. Perform emergency cleaning procedure.
2. Perform Internal Quality Check with CCBg.

9.2.3 Peaks not within the gate

The peak is not within the gate “FL2_1”.

Remedy

Please contact your local Sysmex representative.

10 Disposal

WARNING

Hazardous waste

Liquid waste from the device may contain hazardous substances.

- Read and understand product-specific Safety Data Sheets (SDSs) and safety precautions provided by the manufacturers of chemical substances used before disposing chemical waste.
- Always wear appropriate personal protective equipment.
- Dispose of waste in accordance with applicable local regulations.

WARNING

Risk of infection

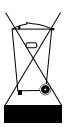
All human-sourced materials and any objects coming into contact with those materials have the potential to transmit infections.

- Always wear appropriate personal protective equipment.
- Keep your working area clean and follow procedures for weekly cleaning and disinfection described in this manual.
- Dispose of all materials in accordance with applicable local regulations.

10.1 Disposal of device

Please contact your local Sysmex representative if you plan to decommission the device. Only authorised service personnel is allowed to decommission the device.

For disposal of the device, follow applicable local regulations. The device must be considered potentially infectious and therefore needs to be specially treated before disposal.



Electrical and electronic equipment must be disposed of separately from municipal waste.

10.2 Disposal of consumables and reagents

Generally, all components and fluids must be disposed of in accordance with local regulations and according to procedures established for your laboratory.

All human-sourced materials and any objects coming into contact with those materials must be considered potentially infectious. This includes disposable accessories like sample tubes, disposable gloves and pipette tips.

Biohazardous materials may require special handling, and disposal limitations may apply. Follow applicable local regulations.

10.3 Disposal of liquid waste

Liquid waste from the device may contain hazardous substances and must be considered potentially infectious. Liquid waste requires special treatment to reduce protein residuals before disposal. Please follow instructions in section “8.2.3 Empty liquid waste” as well as applicable local regulations.

11 Technical data

11.1 Environmental conditions

INFORMATION

Only use the device indoors.

Item	Specification
Temperature	Transport & Storage: 5 - 50 °C Operation: 15 - 30 °C
Relative humidity	20% - 85% relative, non-condensing
Other	Clinical environment, no vibrations, no direct sunlight, no direct heat, no smoke, no dust
Max. altitude	2000 m above MSL
Minimum work space	Height: 700 mm Width: 800 mm Depth: 800 mm
Ventilation space behind device	Back: 200 mm

11.2 Device

NOTICE

EMC class A

This device is not intended to be used in residential areas and can not ensure adequate protection of radio reception in such environments.

- Use the device according to the environmental conditions stated in chapter 11.1 Environmental conditions.

Item	Specification
GMDN classification	Flow Cytometer

Item	Specification
EMC device classification	DIN EN 55011 (VDE 0875-):2018-05; FCC CFR 47 part 18 subpart C
Dimensions	Width: 325 mm Depth: 265 mm Height: 330 mm
Weight	11.5 kg
Voltage	100 – 240 V ±10 %
Frequency	50/60 Hz
Current consumption	max. 0.3 A | 0.6 A
Power consumption	max. 100 VA
Mains fuse	Thermal fuse T 3.15 A / 250 V, EN 60127-2
Installation / overvoltage category	2 / II
Means of protection (Protection class)	Class I (PE connected)
Pollution degree	2
Degree of protection (according to IEC 60529)	IP 20
Laser class	Closed housing: Laser class 1 Open housing and no protection: Laser class IIIb, authorised service personnel only
Noise level	≤ 66 dBA
Heat dissipation	max. 342 BTU/h
Instrument check	Count Check Beads green
Setup time	Max. 5 minutes
Particle size range	50 µm
Maximum data acquisition speed	15,000 events/s

Item	Specification
Acquisition stop time	Volume-based
Trigger sensors	FL2 (for CD4 absolute and CCBg measurement) FL3 (for CD4% measurement)
Display	Touch display

11.3 Optics

Item	Specification
Laser	532 nm, 30 mW (-12 mW / +20 mW)
Parameters detected	3 independent optical parameters: SSC, FL2, FL3
Filters	Standard setup and filters for SSC, FL2 and FL3
Excitation optics	15 µm x 100 µm at 532 nm, elliptical

11.4 Fluidics

Item	Specification
Vacuum	20 kPa below atmospheric pressure
Vacuum application while device is operational	Internal fluidic system, Waste tubing, Air tubing, Waste bottle
Flow Cuvette	Synthetic quartz flow cuvette with centric flow channel (capillary diameter 350 x 250 µm) for laminar sample transport with Sheath Fluid
Sample Delivery	Computer controlled precision syringe pump for contamination-free sample transport. Built-in vacuum pump (200 mbar) for fluid and sample transport.
Sampling Volume	Continuous up to 1600 µL
	Min. 840 µL (TVAC).
Bottle volume	Waste: 1 L
	Sheath: 1 L

Item	Specification	
Sheath consumption	per start-up cleaning:	31 mL
	per priming:	46 mL
	per sample measurement:	28 mL
	per CCBg measurement:	28 mL
	per shutdown cleaning:	38 mL
	per weekly cleaning:	38 mL

11.5 Computer unit

Item	Specification
Electronics	Real-time acquisition Signal processing amplifiers
Computer	Built-in computer Intel® Celeron® processor 8.4'' TFT LCD colour touch screen (SVGA) Thermal printer (built-in)
Interfaces	1x USB 2.0 1x USB 3.0 2 x Ethernet 1 x RS 232/422/485 1x VGA

11.6 Software

Item	Specification
Software	Windows® software FCS standard list mode format report (automatic calculation) FCS 3.0
Adjusting Gates	Online and offline adjustment Gate position stored within ".fcs" file
Configuration	Saves layout of the measurement (regions, scaling), gain and speed values for default
Report	Automatic calculation of $CD4^+$ and CD4% Internal measurement data storage Printed result output
Max. data file capacity	10000

12 Performance data

NOTICE

Dependency on reagent performance

The performance is heavily dependent on the performance of the reagents used with the device.

- Read the associated labelling carefully before using the device. This includes all provided labelling of reagents, disposables and consumables used with this device.
- For analytical and clinical performance parameter please refer to the Instructions for Use of the CD4 and CD4% easy count kit.

12.1 Carryover

Instrument carryover is ≤ 2%.

13 Annex

13.1 Abbreviations

Abbreviation	Explanation
.fcs	Flow cytometry standard (file extension)
µL	Measure of volume (Microliter)
µm	Measure of length (Micrometer)
A	Measure of current (Ampere)
AC	Alternating current
BTU/h	Measure of heat (British heat unit per hour)
CCBg	Count Check Beads green
CD	Cluster of differentiation (T-cell surface structure)
dBA	Measure of level (Decibel, a-weighted)
EMC	Electromagnetic compatibility
FL	Fluorescent light
GMDN	Global Medical Device Nomenclature
HIV	Human immunodeficiency virus
Hz	Unit of frequence (Hertz)
IFU	Instructions for Use
IP	Code (International Protection)
kg	Measure of weight (Kilogram)
kPa	Measure of pressure (Kilopascal)
L	Measure of volume (Liter, sometimes as “L”)
LoD	Limit of detection
mAb	Monoclonal antibody
max.	Maximum / maximal
min.	Minimum / minimal

Abbreviation	Explanation
mL	Measure of volume (Milliliter)
mm	Measure of length (Millimeter)
MSL	Mean sea level
mW	Measure of power (Milliwatt)
nm	Measure of length (Nanometer)
PMT	Photomultiplier tube
PPE	Personal protective equipment
SSC	Side scatter
TFT LCD	Thin-film-transistor liquid-crystal display
ToC	Table of contents
ToF	Table of figures
TVAC	True Volumetric Absolute Counting
V	Measure of voltage (Volt)

13.2 Index

2.4 Cleaning solution IFU (product code 04-4017)

1 Identification of the IVD reagent

Name	Cleaning Solution
Ref. No.	04-4017
UDI-DI	04250878904795
Content	250 mL, ready to use

2 Intended purpose

IVD For In Vitro Diagnostic Use.

Cleaning Solution is intended to clean the sample pathway of Sysmex Partec clinical flow cytometers. Cleaning Solution is ready to use and will be fed to the instrument via sample port manually or via automated loading system. Cleaning Solution is a general laboratory accessory solution and does not provide any diagnostic information.

Handling with Cleaning Solution is restricted to lab technicians and trained FCM operators.

3 Principle of the procedure

The product, as an accessory solution for flow cytometry, is used for cleaning the sample pathway of Sysmex Partec clinical flow cytometers.

For further information refer to the Instructions for Use of the flow cytometer.

4 Storage and shelf life

4.1 Unopened product

Store Cleaning Solution at 18-30 °C, do not freeze or expose to elevated temperatures. Under these storage conditions the reagent will be stable until the expiration date printed on its label. Do not use the reagent beyond its shelf life.

4.2 Product after first opening

The shelf life after first opening is the same as the shelf life for unopened reagent if stored at stated storage conditions and used according to the Instructions for Use (IFU).

5 Components

Cleaning Solution is an aqueous solution without hazardous components. For further information refer to the Safety Data Sheet.

6 Evidence of deterioration

Cleaning Solution is a clear green liquid. Do not use Cleaning Solution after appearance of any kind of turbidity or contamination.

For questions regarding the performance or quality of the product received, please contact your local Sysmex representative.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user is located.

7 Precaution and warnings

Appropriate safety precautions and handling procedures must be applied in accordance with applicable laws and regulations.

Refer to the Safety Data Sheet (SDS) for a complete list of warnings and precautions.

8 Additional required equipment

Instrument:	Sysmex Partec clinical flow cytometer, such as the CyFlow™ Counter (Ref. No. CY-S-3023) For further information, please refer to the instructions for use (IFU) of the flow cytometer.
Laboratory equipment:	A calibrated pipette and pipette tips Sample tube(s) compliant with the flow cytometer Personal protective equipment

9 Disposal

Disposal procedure should meet requirements of applicable local regulations.

10 Manufacturer

Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz
Germany

Phone +49 3581 8746 0
Fax +49 3581 8746 70
info@sysmex-partec.com
www.sysmex-partec.com

11 Symbols

REF	Reference number		Manufacturer	LOT	Batch code
	Temperature limit	IVD	In vitro diagnostic medical device		Use-by date
	Consult instructions for use	CE	CE mark	UDI	Unique device identifier

12 Date of issue or revision

Rev.: 001 CN 2181
Rev. date: 15-12-2021
Doc. No.: 04-4017 IFU GB EN

2.5 Count check beads green IFU (product code 05-4026)

1 Identification of the IVD reagent

Name	Count Check Beads green
Ref. No.	05-4026
UDI-DI	04250878904825
Content	2 x 25 mL, ready to use

2 Intended Purpose

IVD For In Vitro Diagnostic Use.

Count Check Beads green is a dedicated concentrated solution of suitable beads, to be used for a quantitative quality control measurement. It is recommended for the daily quality control of Sysmex Partec clinical flow cytometers, equipped with green excitation light source. It is ready to use and will be fed to the instrument via sample port manually or via automated loading system. Count Check Beads green is a control material and does not provide any diagnostic information.

Handling with Count Check Beads green is restricted to lab technicians and trained FCM operators.

3 Principle of the procedure

Count Check Beads green, as a control material, is used for a quantitative quality control measurement of Sysmex Partec clinical flow cytometers by enumeration of beads with a dedicated concentration within the Count Check Beads green solution. Shake the Count Check Beads green bottle thoroughly for 15 seconds prior use.

For further information refer to the Instructions for Use of the flow cytometer.

4 Storage and shelf life

4.1 Unopened product

Store the reagent at 2-8 °C in the dark. Do not freeze or expose the reagents to elevated temperatures and keep it away from direct sunlight. Under these conditions the reagent will be stable until the expiration date printed on its label. Do not use the reagent beyond its shelf life.

4.2 Product after first opening

The shelf life after first opening is the same as the shelf life for unopened reagent if stored at stated storage conditions and used according to the Instructions for Use (IFU).

5 Components

Count Check Beads green are beads in an aqueous solution.

6 Evidence of deterioration

Count Check Beads green are a clear liquid. Do not use Count Check Beads green after appearance of any kind of turbidity or contamination.

For questions regarding the performance or quality of the product received, please contact your local Sysmex Representative.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user is located.

7 Precautions and warnings

Appropriate safety precautions and handling procedures must be applied in accordance with applicable laws and regulations.

Refer to the Safety Data Sheet (SDS) for a complete list of warnings and precautions.

8 Additional required equipment

Instrument:	Sysmex Partec clinical flow cytometer, such as the CyFlow™ Counter (Ref. No. CY-S-3023)
	For further information, please refer to the instructions for use (IFU) of the flow cytometer.
Laboratory equipment:	A calibrated pipette and pipette tips
	Sample tube(s) compliant to the flow cytometer
	Personal protective equipment

9 Disposal

Disposal procedure should meet requirements of applicable local regulations.

10 Manufacturer

Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz
Germany

Phone +49 3581 8746 0
Fax +49 3581 8746 70
info@sysmex-partec.com
www.sysmex-partec.com

11 Symbols

REF	Reference number		Manufacturer	LOT	Batch code
	Temperature limit	IVD	In vitro diagnostic medical device		Use-by date
	Consult instructions for use	CE	CE mark	UDI	Unique device identifier
	Keep away from sunlight	CONC	Concentration of beads		Fragile, handle with care

12 Date of issue or revision

Rev.: 001 CN 2180
Rev. date: 30-11-2021
Doc. No.: 05-4026 IFU GB EN

2.6 Decontamination solution IFU (product code 04-4018)

1 Identification of the IVD reagent

Name	Decontamination Solution
Ref. No.	04-4018
UDI-DI	04250878904801
Content	250 mL, ready to use

2 Intended purpose

IVD For In Vitro Diagnostic Use.

Decontamination Solution is a solution to clean sample pathways of Sysmex Partec clinical flow cytometers by reducing residual protein. It is ready to use and will be fed to the instrument via sample port manually or via automated loading system. Decontamination Solution is a general laboratory accessory solution and does not provide any diagnostic information.

Handling with Decontamination Solution is restricted to lab technicians and trained FCM operators.

3 Principle of the procedure

The product, as an accessory solution for flow cytometry, is used for cleaning the sample pathway of Sysmex Partec clinical flow cytometers by enzymatic reduction of proteins.

For further information refer to the Instructions for Use of the flow cytometer.

4 Storage and shelf life

4.1 Unopened product

Store Decontamination Solution at 18-30 °C, do not freeze or expose to elevated temperatures. Under these storage conditions the reagent will be stable until the expiration date printed on its label. Do not use the reagent beyond its shelf life.

4.2 Product after first opening

The shelf life after first opening is the same as the shelf life for unopened reagent if stored at stated storage conditions and used according to the Instructions for Use (IFU).

5 Components

Decontamination Solution is an aqueous solution without hazardous components. For further information refer to the Safety Data Sheet.

6 Evidence of deterioration

Decontamination Solution is a clear violet liquid. Do not use Decontamination Solution after appearance of any kind of turbidity or contamination.

For questions regarding the performance or quality of the product received, please contact your local Sysmex representative.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the component authority of the Member State in which the user is located.

7 Precaution and warnings

Appropriate safety precautions and handling procedures must be applied in accordance with applicable laws and regulations.

Refer to the Safety Data Sheet (SDS) for a complete list of warnings and precautions.

8 Additional required equipment

Instrument:	Sysmex Partec clinical flow cytometer, such as the CyFlow™ Counter (Ref. No. CY-S-3023) For further information, please refer to the instructions for use (IFU) of the flow cytometer.
Laboratory equipment:	A calibrated pipette and pipette tips Sample tube(s) compliant with the flow cytometer Personal protective equipment

9 Disposal

Disposal procedure should meet requirements of applicable local regulations.

10 Manufacturer

Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz
Germany

Phone +49 3581 8746 0
Fax +49 3581 8746 70
info@sysmex-partec.com
www.sysmex-partec.com

11 Symbols

REF	Reference number		Manufacturer	LOT	Batch code
	Temperature limit	IVD	In vitro diagnostics medical device		Use-by date
	Consult instructions for use	CE	CE mark	UDI	Unique device identifier

12 Date of issue or revision

Rev.: 001 CN 2182
Rev. date: 15-12-2021
Doc. No.: 04-4018 IFU GB EN

2.7 Hypochlorite solution IFU (product code 04-4019)

1 Identification of the IVD reagent

Name	Hypochlorite Solution
Ref. No.	04-4019
UDI-DI	04250878904818
Content	250 mL, ready to use

2 Intended purpose

IVD For In Vitro Diagnostic Use.

Hypochlorite Solution is intended to clean the sample pathway by reducing possibly remaining blood and/or protein residuals from prior sample measurements in Sysmex Partec clinical flow cytometers. Hypochlorite Solution is ready to use and will be fed to the instrument via sample port manually or via automated loading system. Hypochlorite solution is an accessory solution and does not provide any diagnostic information.

Handling with Hypochlorite Solution is restricted to lab technicians and trained FCM operators.

3 Principle of the procedure

Hypochlorite solution, as an accessory solution for flow cytometry, is used to reduce possible residual proteins in the sample pathway of Sysmex Partec clinical flow cytometers after measurement.

For further information refer to the Instructions for Use of the flow cytometer.

4 Storage and shelf life

4.1 Unopened product

Store Hypochlorite Solution at 18-30 °C, do not freeze or expose to elevated temperatures. Under these storage conditions the reagent will be stable until the expiration date printed on its label. Do not use the reagent beyond its shelf life.

4.2 Product after first opening

The shelf life after first opening is the same as the shelf life for unopened reagent if stored at stated storage conditions and used according to the Instructions for Use (IFU).

5 Components

Hypochlorite Solution is an aqueous solution containing < 1.00 wt% of sodium hypochlorite (CAS no. 7681-52-9).

6 Evidence of deterioration

Hypochlorite Solution is a clear liquid. Do not use Hypochlorite Solution after appearance of any kind of turbidity or contamination.

For questions regarding to the performance or quality of the product received, please contact your local Sysmex representative.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the component authority of the Member State in which the user is located.

7 Precautions and warnings

Appropriate safety precautions and handling procedures must be applied in accordance with applicable laws and regulations.

Refer to the Safety Data Sheet (SDS) for a complete list of warnings and precautions.

7.1 Warning symbols

GHS05
Corrosive

7.2 Signal word

DANGER

7.3 Hazards

H314	Causes severe skin burns and eye damage.
H412	Harmful to aquatic life with long lasting effects.

7.4 Precautions

P260	Do not breathe mist/vapours/spray.
P264	Wash thoroughly after handling.
P280	Wear protective gloves/eye protection.
P303+P361+P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER/doctor.
P501	Dispose of contents/container to a facility in accordance with local and national regulations.

8 Additional required equipment

Instrument:	Sysmex Partec clinical flow cytometer, such as the CyFlow™ Counter (Ref. No. CY-S-3023)
	For further information, please refer to the instructions for use (IFU) of the flow cytometer.
Laboratory equipment:	A calibrated pipette and pipette tips
	Sample tube(s) compliant to the flow cytometer
	Personal protective equipment

9 Disposal

Disposal procedure should meet requirements of applicable local regulations.

10 Manufacturer

Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz
Germany

Phone +49 3581 8746 0
Fax +49 3581 8746 70
info@sysmex-partec.com
www.sysmex-partec.com

11 Symbols

REF	Reference number		Manufacturer	LOT	Batch code
	Temperature limit	IVD	In vitro diagnostic medical device		Use-by date
	Consult instructions for use	CE	CE-mark	UDI	Unique device identifier

12 Date of issue or revision

Rev.: 001 CN 2179
Rev. date: 08-11-2021
Doc. no.: 04-4019 IFU GB EN

2.8 Sheath fluid IFU (product code 04-4016)

1 Identification of the IVD reagent

Name	Sheath Fluid
Ref. No.	04-4016
UDI-DI	04250878904788
Content	5000 mL container incl. tap, ready to use

2 Intended Purpose

IVD For In Vitro Diagnostic Use.

Sheath Fluid is intended to ensure that the sample, fed to the Sysmex Partec clinical flow cytometer, will run under hydrodynamic focusing. Sample run, and Sheath Fluid consumption will be controlled via FCM software automatically. Sheath Fluid is a general laboratory accessory solution and does not provide any diagnostic information. Handling with Sheath Fluid is restricted to lab technicians and trained FCM operators.

3 Principle of the procedure

Sheath Fluid as an accessory solution for flow cytometry will be passed through the fluidic system of the flow cytometer via vacuum pressure. Once the Sheath Fluid is running at laminar flow, the sample flow will be injected into the center of the stream, at a slightly higher pressure. The principles of hydrodynamic focusing cause the cells to align, single file in the direction of flow.

For further information, please refer to the Instructions for Use (IFU) of your flow cytometer.

4 Storage and shelf life

4.1 Unopened product

Store at 18 - 30°C, do not freeze or expose to elevated temperatures. Under these storage conditions the reagent will be stable until the expiration date printed on its label. Do not use the reagent beyond its shelf life.

4.2 Product after first opening

Sheath Fluid will be stable at least 7 days after first use. Always use a new clean tap after the Sheath Fluid container was first opened. Close the tap after each use, to avoid contamination.

5 Components

5000 mL aqueous solution

6 Evidence of deterioration

Sheath Fluid is a clear liquid. Do not use Sheath Fluid after appearance of any kind of turbidity or contamination.

For questions regarding to the performance or quality of the product received, please contact your local Sysmex Representative.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user is located.

7 Precautions and warnings

Appropriate safety precautions and handling procedures must be applied in accordance with applicable laws and regulations.

Refer to the Safety Data Sheet (SDS) for a complete list of warnings and precautions.

8 Additional required equipment

Instrument:	Sysmex Partec clinical flow cytometer, such as the CyFlow™ Counter (Ref. No. CY-S-3023)
	For further information, please refer to the instruction for use (IFU) of the flow cytometer.
Laboratory equipment:	A calibrated pipette and pipette tips
	Sample tube(s) compliant to the flow cytometer
	Personal protective equipment

9 Disposal

Disposal procedure should meet requirements of applicable local regulations.

10 Manufacturer

Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz
Germany

Phone +49 3581 8746 0
Fax +49 3581 8746 70
info@sysmex-partec.com
www.sysmex-partec.com

11 Symbols

REF	Reference number		Manufacturer	LOT	Batch code
	Temperature limit	IVD	In vitro diagnostic medical device		Use-by date
	Consult instructions for use	CE	CE mark	UDI	Unique device identifier
	Stacking limit by number		This way up		Use no hooks
	Keep dry				

12 Date of issue or revision

Rev.: 001 CN 2151
Rev. date: 21-09-2021
Doc. No.: 04-4016 IFU GB EN