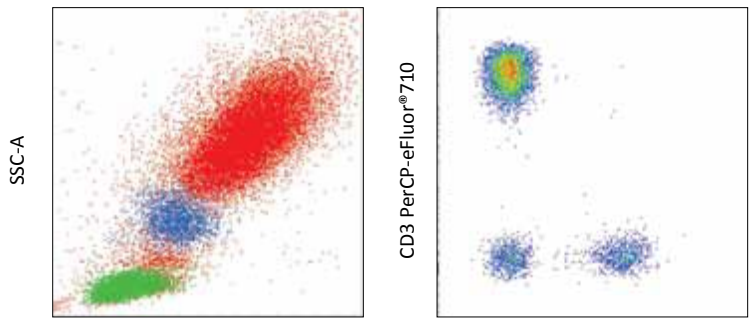
# Flow Cytometry Buffers & Solutions

Optimal detection of cellular antigens by flow cytometry requires the use of optimized staining buffers. eBioscience provides appropriate staining and cell preparation buffers regardless of whether your target is secreted, nuclear, or on the cell surface.

## 1-Step Fix/Lyse Solution (10X)

eBioscience 1-Step Fix/Lyse Solution simplifies your analysis of human blood. This solution lyses red blood cells (RBCs) after staining peripheral blood with fluorochrome conjugated antibodies, and leaves behind stained and fixed leukocytes, all in one step.

1-Step Fix/Lyse Solution Advantages Include:
- RBC lysis and fixation of samples in only 15 minutes
- Allows temporary storage of fixed and stained samples
- Eliminates the need for gradient centrifugation separation




### 1-Step Fix/Lyse Solution (10x)

*Normal human peripheral blood cells were stained with FITC anti-human CD45, PerCP-eFluor® 710 anti-human CD3, and eFluor® 450 anti-human CD19, and then incubated with eBioscience 1-Step Fix/Lyse Solution (cat.no. 00-5333) for 15 minutes at room temperature. Cells were centrifuged,washed once in flow staining buffer, and then analyzed.*

*Left: CD45+ granulocytes (red), monocytes (blue), and lymphocytes (green)can be seen in the forward vs. side scatter plot of total viable cells.*

*Right: Analysis of cells gated on CD45+ events.*

New products are launched regularly. Discover more at www.eBioscience.com.

| Name | Application | Cat. No. |
|---|---|---|
| 1-step Fix/Lyse Solution (10X) | FC | 00-5333 |
| 10X RBC Lysis Buffer (Multi-species) | FC, FA | 00-4300 |
| 1X RBC Lysis Buffer | FC | 00-4333 |

When performing intracellular staining for flow cytometry the selection of buffers used for fixation and permeabilization has a significant impact on the quality and accuracy of data. eBioscience provides optimized buffer solutions for nuclear factors, transcription factors, and cytosolic and secreted proteins. The table below summarizes which buffer system is appropriate for target antigens in various cellular locations.

# Intracellular Staining Buffer Selection Guide

| Target Antigen | eBioscienceFoxp3 / Transcription Factor Staining Buffer Set<br><br>Cat. No. 00-5523 | eBioscience Intracellular Fixation & Permeabilization Buffer (plus Brefeldin A)<br>Cat. No. 88-8823<br>eBioscience Intracellular Fixation & Permeabilization Buffer Set<br>Cat. No. 88-8824 |
|---|---|---|
| | The Foxp3 Staining Buffer Set have been formulated and optimized for staining with the the Foxp3 antibodies, FJK-16s, NRRF-30, PCH101, 236A/E7 and eBio7979 monoclonal antibodies. It has also been shown to work for other transcription factors such as Nanog, Tbet, Gata-3, Ror gamma as well as cytokines.<br><br>Please see our Frequently Asked Questions regarding the usage of eBioscience Foxp3 antibodies and staining reagents. | Description: The eBioscience Fixation and Permeabilization Kit is designed for use in preparation of living cells for intracellular staining and flow cytometric analysis. A protein transport inhibitor (Brefeldin A) is included for treatment of cells during culture – to cause accumulation of cytokine protein at the Golgi and enhance resulting staining. A fixation buffer is used to cross link, stabilize, and 'fix' the cell membrane, while the permeabilization buffer is used to reversibly permeabilize the cell membrane so the staining antibodies can enter the cell effectively. |
| **Transcription Factors** | **Yes** | **No** |
| **Nuclear Proteins** | **Yes** | **Not tested** |
| **Cytokines (Secreted Proteins)** | **Yes‡** | **Yes** |
| **Cytoplasmic Proteins** | **Not tested** | **Yes** |

**‡ More information by visiting our web site :** Antibody Fixation and Intracellular Staining Considerations

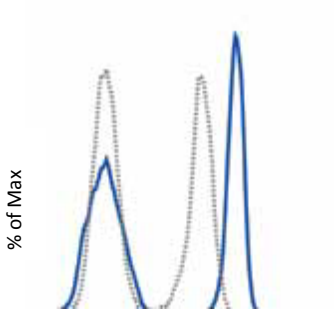
Products and Catalog Numbers

| Name | Application | Cat. No. |
|---|---|---|
| 1-step Fix/Lyse Solution (10X) | FC | 00-5333 |
| 10X RBC Lysis Buffer (Multi-species) | FC, FA | 00-4300 |
| 1X RBC Lysis Buffer | FC | 00-4333 |
| Brefeldin A Solution (1000X) | FC | 00-4506 |
| Cell Stimulation Cocktail (500X) | FC, ELISA, FA | 00-4970 |
| Cell Stimulation Cocktail (plus protein transport inhibitors) (500X) | FC, FA | 00-4975 |
| Human Fc Receptor Binding Inhibitor Functional Grade Purified | FC | 16-9161 |
| Human Fc Receptor Binding Inhibitor Purified | FC | 14-9161 |
| Intracellular Fixation & Permeabilization Buffer (plus Brefeldin A) (previously named IC Fixation & Permeabilization Buffer) | FC | 88-8823 |
| Fixation/Permeabilization Concentrate | FC | 00-5123 |
| Fixation/Permeabilization Diluent | FC | 00-5223 |
| Flow Cytometry Staining Buffer | FC | 00-4222 |
| Foxp3 Fixation/Permeabilization Concentrate and Diluent | FC | 00-5521 |
| Foxp3 / Transcription Factor Staining Buffer Set | FC | 00-5523 |
| IC Fixation Buffer | FC | 00-8222 |
| Intracellular Fixation & Permeabilization Buffer Set | FC | 88-8824 |
| Monensin Solution (1000X) | FC | 00-4505 |
| OneComp eBeads | FC | 01-1111 |
| Permeabilization Buffer (10X) | FC | 00-8333 |
| Protein Transport Inhibitor Cocktail (500X) | FC, FA | 00-4980 |

More information and protocols by visiting our web-site : Flow Cytometry (FACS) Protocols

# Fixation Considerations For Violet Laser Dyes

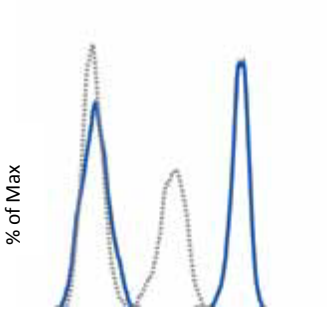
The ability of fluorophore conjugated reagents to retain fluorescence performance after fixation is a critical parameter necessary to accommodate all work flow scenarios. Some generalizations regarding fluorophore performance after fixation can be made, but clonespecific performance should be determined empirically. In the data shown below, human PBMCs were stained with eFluorR 450, eFluorR 605NC or eFluorR 650NC conjugated to anti-CD4 or anti-CD3 prior to fixation. The blue histogram represents staining of unfixed cells and the grey dotted histogram represents staining of fixed cells.



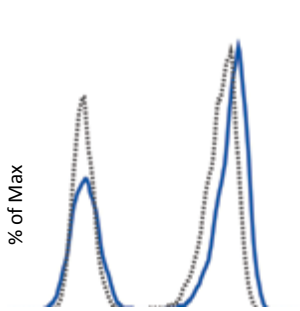

CD4 (SK3) eFluor®450

## eFluor® 450

Under conditions where the cell sample was left in 2% Paraformaldehyde(PF) for 48 hours, there is an approximately 60% reduction in MFIfollowing fixation. We recommend a shorter exposure to fixative, and have seen minimal loss of fluorescence when cells are exposed to fixativefor ≤ 1 hour.




CD4 (OKT4) eFluor®605NC



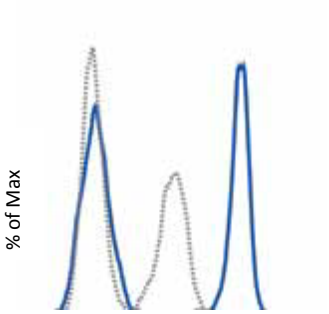

CD4 (OKT4) eFluor®605NC

## eFluor® 605NC and eFluor® 650NC

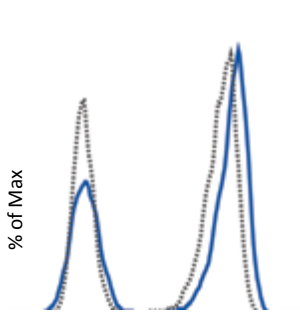
The composition of nanocrystals makes these reagents much more sensitive to fixation conditions than conventional organic dyes or fluorescent proteins.

This can be observed in the data shown here for both the 605NC and 650NC (data boxes on left for each pair) when exposed to 2% paraformaldehyde for 48 hours.

We have found that limiting the time of fixation to ≤ 30 minutes results in minimal loss of fluorescence (data boxes on right for each pair).



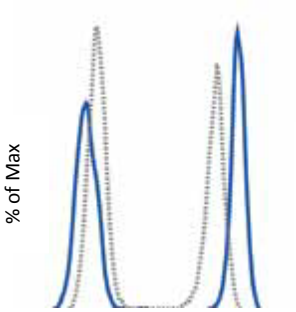

CD4 (RPAT4) eFluor®650NC



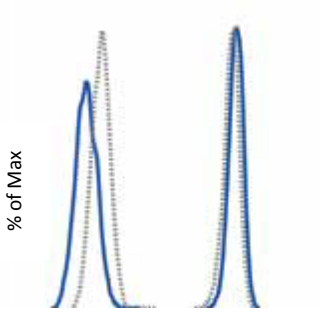

CD3 (RPAT4) eFluor®650NC

# Fixation Considerations For Blue Laser Dyes

The data shown on page 10 represents an assessment of fluorophore performance after fixation. The ability of fluorophore conjugated reagents to retain fluorescenceperformance after fixation is a critical parameter necessary to accommodate all work flow scenarios. Some generalizations regarding fluorophore performance after fixation can be made, but clone specific performance should be determined empirically. In the examples shown below, human PBMCs were stained with anti-CD4 reagents conjugated to our fluorophores for the blue laser prior to fixation. The blue histogram represents staining of unfixed cells and the grey dotted histogram represents staining of fixed cells.



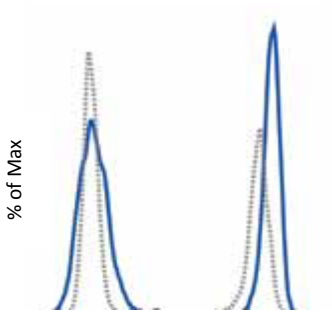

CD4 (SK3) FITC




CD4 (OKT4) AlexaFluor®488

## FITC and Alexa Fluor® 488

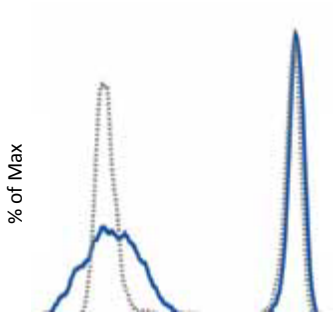
Both of these small molecule fluorescent dyes are amenable to fixation and although FITC generally shows some decrease in fluorescence intensity following fixation, in most cases the resolution of the positive population is unaffected.



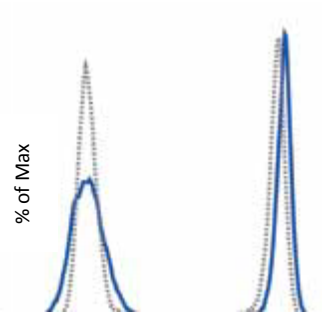

CD4 (SK3) PE

## PE

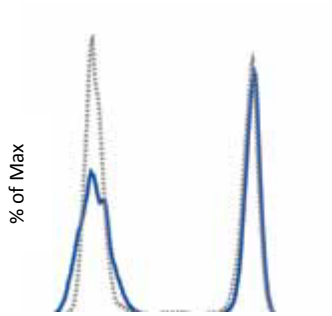
Fixation of PE conjugates will generally result in a 30-40% decrease in MFI. However, because the stain index of PE is so high and produces superb resolution of positive and negative populations, this decrease in MFI after fixation has little impact on interpretation of data obtained from cells stained with PE conjugated reagents.




CD4 (OKT4) PE-Cy5



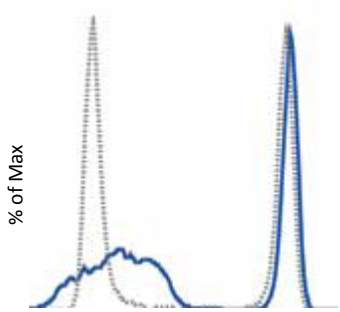

CD4 (SK3) PerCP-eFluor®710




CD4 (RPA-T4) PerCP-Cy5.5

## PE-Cy5, PerCP-eFluor® 710, and PerCP-Cy5.5

All three tandem dyes perform well when subjected to fixation with virtually no loss of fluorescent signal.



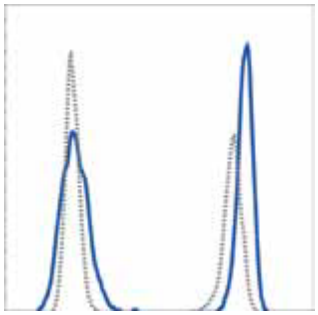

CD4 (RPA-T4) PE-Cy7

## PE-Cy7

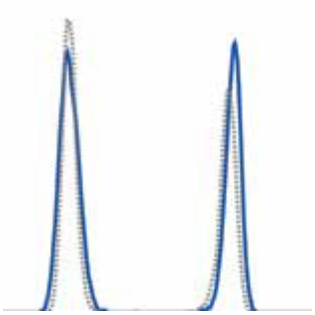
eBioscience PE-Cy7 has been optimized for performance and has virtually no loss of fluorescent signal when subjected to fixation. Additionally, fixation of this tandem dye does not increase the amount of compensation required from the PE detector.

# Fixation Considerations For Red Laser Dyes

The following data are presented as an example of fluorophore performance following fixation with paraformaldehyde. The ability of fluorophore conjugated reagents to retain fluorescence performance after fixation is necessary to provide flexibility for a variety of work flow scenarios. Some generalizations regarding fluorophore performance after fixation can be made, but clone-specific performance should be determined empirically.
In the examples shown here, human PBMCs were stained with either anti-CD4 reagents(APC, Alexa FluorR 647 and Alexa FluorR 700) or anti-CD8 (APC-eFluorR 780) prior to fixation. The blue histogram represents staining of unfixed cells and the grey dotted histogramrepresents staining of fixed cells.



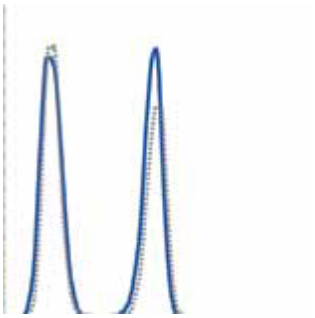
CD4 (SK3) APC



CD4(OKT4) Alexa Fluor®647

## APC, eFluor® 660 and Alexa Fluor® 647

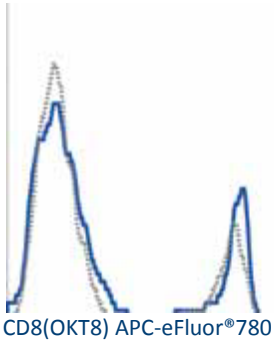
All of these fluorophores are amenable to fixation. While APC shows a 38% reduction in MFI in this example, the resolution of the positive population from the negative, as calculated by the stain index, is unaffected.



CD4(OKT4) Alexa Fluor®700

## Alexa Fluor® 700

This small organic molecule exhibits no loss of fluorescent signal following fixation.



CD8(OKT8) APC-eFluor®780

## APC-eFluor® 780

This tandem dye retains its fluorescent signal following fixation. Additionally,fixation does not increase the amount of compensation required from the APC detector.

**More information by visiting our website :** Antibody Fixation Considerations