VIR*Type* SARS-CoV-2 (L452R)

TEST KIT FOR SARS-COV-2 VARIANT DETECTION CARRYING L452R MUTATION USING RT-REAL-TIME PCR FROM FOOD & ENVIRONMENTAL SURFACES AND WASTEWATER

Cat. No. 5728401601

For 48 RT-qPCR reactions

VIR*Seek*

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1 INTRODUCTION

Coronavirus disease (COVID-19) is an infectious disease caused by a newly discovered coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The main route of transmission is primarily from person-to-person via respiratory droplets from coughs and sneezes. The potential indirect route of transmission by touching surfaces is discussed, as data have been generated proving a survival of SARS-CoV-2 on surfaces such as stainless steel for up to 72 hours in a laboratory setup (see reference 1). These findings render testing of environmental, as well as food surfaces, reasonable and knowledge of the presence of SARS-CoV-2 genomic RNA would enable businesses, individuals, state agencies to take adequate decisions in regards to cleaning and/or decontamination as well as measure the effectiveness of cleaning.

Genetic variants of SARS-CoV-2 have been emerging and circulating around the world throughout the COVID-19 pandemic, These SARS-CoV-2 variants often carry multiple spike protein mutations as well as mutations in other genomic regions. The spike protein is key for viral entry and for vaccine development. The WHO and national authorities such as the US government jointly developed a variant classification scheme that defines different classes of SARS-CoV-2 variants, depending on the presence of those defined genetic markers (variant of interest) or variants for which clear evidence in either viral transmission or neutralizing capacities by antibodies is given (variants of concern or high concern) (see reference 2).

The VIR*Type* SARS-CoV-2 (L452R) kit detects one single point mutation which result in the exchange of the amino acid Leucine to Arginine in the spike protein at position 452. This mutation at position 452 is found in the California lineages B.1.429 and B1.427 – both classified in the US as variants of concern. This single point mutation at position 452 is also found in lineage B. 1.526.1 initially identified in New York in October 2020 and classified as a variant of interest as well as lineage B.1.617, the recently emerging lineage in India.

The primer / probe combination of this RT-qPCR system is highly specific for the single point mutation at position 452 of the spike genome region in SARS-CoV-2 variant lineages and does not cross-react with the S-gene region of SARS-CoV-2 wild type strain or other mutations in the spike protein.

The VIR*Type* SARS-CoV-2 (L452R) kit provides all reagents for the rapid detection of the California lineages B.1.429 and B.1.427 as well as lineage B.1.526.1 and B.1.617 on food and environmental surfaces as well as wastewater via RT-qPCR. Single point mutation referring to the L➔R amino acid exchange specific for those variants is detected in the FAM™ channel. As a reference, the respective wild type RdRP-gene region is detected in the HEX™ channel helping to confirm the presence of SARS-CoV-2.

The VIR*Type* SARS-CoV-2 (L452R) kit is validated for use with the Agilent AriaMx™, Bio-Rad CFX96 Touch™ and the CFX96 Touch™ Deep Well PCR platforms.

The kit is intended to be used by analytical laboratories for wastewater or environmental surface samples as part of quality control / quality assurance testing, (e.g. virological monitoring of production processes) or food surface testing, however transmission via food surfaces is currently under investigation and has not been confirmed as a route of exposure.

The kit is not intended for clinical diagnostics and should therefore be regarded as “For Research Use Only”.

1.1 Test Principle

Primarily, an adequate protocol for sampling of viral material e.g. from environmental surfaces, such as described in the PathoSwab 50 or from food surfaces acc. to ISO 15216, followed by a suitable RNA extraction approach, e.g. VIR*Seek* RNA*Extractor* (see section 1.4), is required for these sample types.

Subsequently, isolated viral RNA is analysed in a respective RT-qPCR assay, where the viral RNA is transcribed into cDNA (reverse transcription (RT)) in the first step and is subsequently amplified by real-time PCR. DNA amplification and detection methods take advantage of the nucleotide sequence conservation found in viral genomes that allow highly specific and sensitive detection of pathogenic viruses.

Therefore, a highly specific primer/probe PCR system is recommended for a screening approach, in order to sensitively detect SARS-CoV-2 positive samples. This can be achieved by using the VIR*Seek* SARS-CoV-2 Mplex kit, which detects two targets on the N-gene sequence, including the possibility to determine extraction efficiency by using an extraction control in the Cy5™ channel.

The VIR*Type* SARS-CoV-2 (L452R) assay can also be used as a stand-alone assay, as the RdRP-gene is also detected in the HEX™ channel helping to identify SARS-CoV-2, in addition to the specific single point mutation.

The VIR*Seek* SARS-CoV-2 Mplex kit and the VIR*Type* SARS-CoV-2 (L452R) kit can be run using the same rapid thermal profile on the same validated thermal cyclers.

1.2 Components of the Kit

Please pay attention to the storage condition and the maximum number of freeze/thaw cycles.

- 1x **Oligo Mix VIR*Type* SARS-CoV-2 (L452R)**, vials with yellow white caps, contains primers /probes for L452R single point mutation in S-Gene region, 159 µL, store at -20 °C ± 2 °C, do not freeze / thaw more than 3 times.
- 1x **Basic Mix VIR*Type* SARS-CoV-2 (L452R)**, vials with white caps, 265 µL, store at -20 °C ± 2 °C, do not freeze / thaw more than 3 times.
- 1x **Positive Control VIR*Type* SARS-CoV-2 (L452R)**, vial with red cap, 100 µL, store at -20 °C ± 2°C, do not freeze / thaw more than 3 times.
- 1x **Negative Control VIR*Type* SARS-CoV-2 (L452R)**, vial with transparent cap, 500 µL, store at -20 °C ± 2 °C.

1.3 Additional Equipment, Consumables and Reagents Required

Equipment

- 1x **Stepper pipette (1 mL),** (e.g. HandyStep® S (Brand®), Eurofins GeneScan Technologies GmbH, cat. no. 5617703401).
- 1x **Single channel pipette (1 mL, 100 µL),** (e.g. Transferpette® S 100 - 1000 µL (Brand®), Eurofins GeneScan Technologies GmbH, cat. no. 5617703301).
- 1x **Single channel pipette (100 µL, 10 µL),** (e.g. Transferpette® S 10 - 100 µL (Brand®), Eurofins GeneScan Technologies GmbH, cat. no. 5617703201).
- 1x **Single channel pipette (up to 10 µL),** (e.g. Transferpette® S, 0.5 - 10 µL (Brand®), Eurofins GeneScan Technologies GmbH, cat. no. 5617703101).
- 1x **Cooling block** for 1.5 mL tubes, (e.g. Biozym, BCS-163).
- 1x **96 well cooling block,** (e.g. Blue cooling block 96 well, Eurofins GeneScan Technologies GmbH, cat. no. 5613900501).
- 1x **Vortex mixer,** (e.g. VWR Collection, cat. no. 444-2790).

Centrifuge for microtiter-plates / or -strips - depending on throughput:

- Capacity of 2x 8-well strips: (e.g. Carl Roth GmbH, Rotilabo® centrifuge with butterfly rotor, cat. no. T465.1).
- Capacity of 4x 8-well strips: (e.g. Mini Centrifuge IKA Mini G, cat. no. 5613902601 or VWR, MiniStar silverline cat. no. 521-2844P).
- Capacity of two times 12x 8-well strips: (e.g. Benchmark Scientific, PlateFuge™ microplate microcentrifuge, cat. no. 5613901701).

Real-time PCR Thermocycler:

- Agilent AriaMx™ with FAM™ and Cy5™ filter set (AriaMX Software, up to version 1.5).
- Bio-Rad CFX96 Touch™ (CFX Manager™ Software / CFX Maestro™ Software).
- Bio-Rad CFX96 Touch™ Deep Well (CFX Manager™ Software / CFX Maestro™ Software).

Consumables and material not contained in the kit

- **RNase-free water** (molecular biology grade).
- **Nucleic acid / Nuclease-free pipette tips** with filters, need to be compatible with pipettes used.
- **RNase-free reaction tubes,** 1.5 mL (e.g. DNA LoBind Tubes, Eppendorf, cat. no. 0030108051).
- **PCR plates or strips**, compatible with thermocycler used.
- **Optical 8-caps strip or equivalent seals** (compatible with thermocycler used).
- **RNase decontaminating reagent** (e.g. RNase AWAY® Carl Roth GmbH, cat. no. A998).
- **DNA degrading agent** (e.g. Roti® Nucleic Acid-free, Carl Roth GmbH, cat. no. HP69).
- **Gloves,** powder free.
- **Ice/cooling block**

1.4 Complementary Portfolio of the VIR*Seek* Solution

- **VIR*Seek* SARS-CoV-2 Ident 2**, cat. no. 5728200705
  - RT-qPCR kit with 48 reactions for rapid detection of SARS-CoV-2 specific RdRP sequence in environmental and food surface samples.
- **VIR*Seek* SARS-COV-2 Mplex**, cat. no. 5728201101
  - RT-qPCR kit with 96 reactions for the detection of the N1/N2-gene targets of SARS-CoV-2 in environmental and food surface samples.
- **VIR*Type* SARS-COV-2 Mplex (N501Y, L452R)**, cat. no. 5728401301
  - RT-qPCR kit with 48 reactions for the detection of the N501Y and L452R single point mutations SARS-CoV-2 in food and environmental surface samples as well as in wastewater.
- **VIR*Type* SARS-COV-2 (K417N)**, cat. no. 5728401501
  - RT-qPCR kit with 48 reactions for the detection of the K417N single point mutations SARS-CoV-2 in food and environmental surface samples as well as in wastewater.
- **VIR*Type* SARS-COV-2 (A570D)**, cat. no. 5728401401
  - RT-qPCR kit with 48 reactions for the detection of the A570D single point mutations SARS-CoV-2 in food and environmental surface samples as well as in wastewater.

- **VIR*Seek* Murine Norovirus (MNV) Process Control**, cat. no. 5728200401
  - Murine norovirus spiking material (1 mL) and RT-qPCR kit with 48 reactions for rapid detection of murine norovirus (MNV) process control virus in samples.
  - Also available without Murine norovirus spiking material (cat. no. 5728200801).
- **VIR*Seek* RNA*Extractor***, cat. no. 5524400101
  - Kit for extraction of viral RNA via silica-coated magnetic beads.
- **VIR*Seek* RNA*Extractor* AE1**, cat. no. 5524400801 / 5524400805
  - Kit for 96 automated viral RNA isolations from environmental samples. Validated for Thermo Fisher Scientific KingFisher™ Flex.

2 HOW TO USE THIS PRODUCT

2.1 Important Notes

- Store all reagents as indicated in section 1.2.
- During RT-qPCR set-up:

Keep all reagents on ice ice/cooling block.

Perform all pipetting steps on ice/cooling block.

- Do not use the reagents beyond the expiration dates printed on the labels.
- Never store kit components in the vicinity of samples or post-PCR products.
- Ideally perform RT-qPCR in a UV PCR cabinet.

2.2 General and Safety Precautions

- All samples should be handled with caution, ideally in a bio safety cabinet class II, as they are potentially infectious.
- Viruses should not be handled by pregnant women, children, elderly and immuno-compromised individuals due to the high infection risk and potentially fatal health consequences for this group, in particular for the unborn child in case of pregnant women.
- For more information, please refer to the safety data sheet.

2.3 Working Guidelines

- Comply with Good Laboratory Practice (refer to EN ISO 7218 standard).
- Refer to EN ISO 22174:2005 for the general requirements for the in-vitro amplification of nucleic acid sequences.
- Refer to ISO 15216-2:2019 for virus sampling and extraction from food surfaces.
- For sampling from environmental surfaces, please follow the respective WHO guideline (see reference 3) before proceeding with RNA extraction.
- Perform cleaning protocol (outlined in section 2.5).
- Use DNA-, nuclease-free and sterile lab ware.
- Wear gloves and change frequently.

2.4 RNA Handling – Specific Working Guidelines

It is important to create and maintain an RNase-free environment when working with RNA. RNases are very thermostable enzymes degrading RNA – even in small quantities. Laboratory personnel are the main source for RNase contamination as RNases are expressed in human keratinocytes and are present on skin and hairs.

- Separate the different procedures spatially.
  Ideally use separate rooms for sample preparation and PCR setup laid-out to maintain a strict "one-way-system", thus avoiding cross-contamination in the work stream.
  At least dedicate different areas, equipment and consumables for each procedure.
- Establish a working area, designated as "RNase-free", in which only RNA work is performed. If the RNase-free working area is inside a lab with non-RNase-free working areas, clearly indicate RNase-free parts, e.g. using colour tape.
- Use dedicated RNase-free lab equipment (e.g. pipettes) for RNA-related work. Glassware has to be cleaned and decontaminated before use. For decontamination we recommend baking glassware at >200 °C for ≥4 hours.
- Only use RNase-free filter tips and consumables which are certified to be RNase-free.
- Control high risk areas for DNA / amplicon contamination on a regular basis (swabs / PCR analysis).
- Clean the RT-qPCR working area as described in the cleaning protocol (see section 2.5).
- Wear disposable gloves (latex or vinyl gloves) to prevent contamination with RNases which are present on human skin. Change gloves frequently during the procedure and / or after touching skin, hair, common surfaces etc.
- Wear a lab coat to prevent contamination from clothes.
- Always thaw RNA on a cooling block/ on ice and store RNA at -20 °C or below.
- Handle RT-qPCR enzyme mix as briefly as possible at 0 °C or above. Do not mix reagents from different kits and do not mix reagents from different batches. Return all reagents to 20 °C ±8 °C after usage.
- Store VIR*Type* kit components for RT-qPCR in dedicated areas, and separate from sample storage.
- Only open one tube at a time and always change pipette tips between liquid transfers to avoid cross-contamination.

2.5 Cleaning Protocol

Before commencing work and after completing the work, ensure that the RT-qPCR working area is cleaned as follows:

Cleaning steps	Cleaning protocol
1.	Decontaminate surfaces with Roti® Nucleic Acid-free* or 1 % HCl (hypochlorite acid) to remove DNA / RNA contamination.
2.	Clean the work surfaces and non-disposable laboratory equipment (pipettes, shaker, thermo shaker etc.) with an RNase decontaminating solution* (e.g. RNase AWAY®, Carl Roth, cat. no. A998) to remove RNase contaminations.

** Follow the manufacturer's instructions.*

2.6 Waste disposal

Dispose of any waste which is potentially contaminated with a pathogenic virus according to your internal and local regulations.

For disposal of reagents and chemicals please refer to safety information.

2.7 RT-qPCR

2.7.1 Special Precautions during RT-qPCR Analysis

RT-PCR includes the reverse transcription (RT) of RNA into cDNA. RNA is a molecule which is particularly at risk of degradation due to abundant free RNases in the environment. Prior to RT, special emphasis has to be put on RNase-free environments (see section 2.4).

PCR is an exponential reaction. Therefore, after RT and amplification, the detection of single DNA targets is possible. The extreme sensitivity requires special precautions for handling and equipment. After a successful amplification, several billion amplicons are present in the reaction tube. Each of them might lead to a false positive result when contaminating sample material, i.e. by spreading as aerosols.

2.7.2 PCR Setup

Keep all components (Oligo Mix and Basic Mix) permanently on ice/cooling block during PCR setup.

Prepare final reaction mix fresh each time and immediately before starting the RT-qPCR run.

Calculate required number of reactions and pipette all components (Oligo Mix and Basic Mix) together and mix for the final reaction mix. The final RT-qPCR reaction mix is prepared with an additional 10 % volume.

Frequent freezing and thawing might cause inactivation of the reagents. Do not freeze / thaw kit components more than three times.

Components of final reaction mix	**Amount per reaction**	**e.g. for 10 RT-qPCR reactions (+ 10 %)**
Basic Mix	5 µL	55 µL
Oligo Mix	3 µL	33 µL
Total volume	8 µL	88 µL

Before starting the practical working steps make sure you have switched on the computer, the PCR instrument and ensure the sample layout for the PCR plate is suitably documented and programmed (see below “Plate Setup”).

1. Place PCR plate or strips into the 96-well cooling block which has been cooled at -20 °C.
2. Add 8 µL of final reaction mix to each test well.
3. Add 12 µL VIR*Type* Positive Control (L452R) or VIR*Type* Negative Control respectively to the corresponding wells.
4. Add 12 µL of each sample to the corresponding reaction well of the PCR plate.
5. Use optical caps or foil to seal the PCR plate / strips.
6. Spin down the plate / strips in a centrifuge.
7. Transfer the PCR plate / strips in cooled condition to the RT-qPCR instrument and start the run according to the thermocycler’s instructions.
8. Store samples at -20 °C or below in case PCR needs to be repeated.

Samples and Controls for VIR*Type* SARS-CoV-2 (L452R) Assay

Designation	Volume of reaction mix	Addition of
Test samples	8 µL	12 µL of sample
Positive control (C^+) HEX™ (L452)	8 µL	12 µL of VIR*Type* Positive Control (L452)
Negative control (C^-)	8 µL	12 µL of VIR*Type* Negative Control

Thermal Profile for AriaMx™ and Bio-Rad CFX96 Touch™

1 HOLD	1 HOLD	40 CYCLES	
Reverse transcription	Enzyme activation & Reverse transcriptase inactivation	Denaturation	Annealing & Extension
10 min at 50 °C	3 min at 95 °C	10 sec at 95 °C	30 sec at 60 °C
No data collection	No data collection	No data collection	Data collection

For Bio-Rad CFX96 Touch™ Standard and Deep Well use default ramp rate.

Probe / Detection System

VIR*Type* SARS-CoV-2	Gene variant	Dye
SARS-CoV-2 S-gene	S-gene L452R mutation	FAM™
SARS-CoV-2 RdRP-gene	RdRP-gene	HEX™

3 DATA INTERPRETATION

Data is analysed by using the appropriate software provided by the cycler manufacturer.
For evaluation with the cycler software, we recommend the following settings:

RT-qPCR Thermocycler	Threshold	Baseline
Agilent AriaMX™	Auto[1)]	Adaptive
Bio-Rad CFX96 Touch™	Auto (Check visually)	Baseline Subtracted Curve Fit[2)]
Bio-Rad CFX96 Touch™ Deep Well		Baseline Subtracted Curve Fit[2)]

[1)] *If appropriate, auto calculated threshold with default background based threshold settings can be used: Cycle range: 5 thru 9; Sigma multiplier: 10.*

[2)] *Always apply fluorescence drift correction*

- If the threshold is set incorrectly in automatic mode, adjust it manually.
- For orientation the amplification curve of the positive control should be used.
- The threshold should be set at the beginning of the exponential phase of this curve.

3.1 Export of Raw Data

For raw data export please follow the instruction in the corresponding cycler analysis software.

3.2 Evaluation of Results

3.2.1 Threshold setting

The interpretation of real-time PCR runs depends on automated algorithms that analyse and interpret the measured fluorescence data.

These algorithms are thoroughly validated by the manufacturers of the PCR cyclers and the respective evaluation software and provide reliable results for the vast majority of analyses.

In rare cases, when the automated threshold calculation of the cycler software fails, manual adjustments are reasonable and justified in order to get a correct interpretation of the measured data (e.g. automatic threshold settings might calculate a threshold which is not set in the exponential phase of the amplification curves).

3.2.2 Examples of threshold settings for Agilent AriaMx™ and Bio-Rad CFX™:

In both software platforms the threshold can be adjusted via drag & drop if the automatic threshold calculation fails.

In case of failure of the automatic threshold settings, it is possible to review the curves and set the threshold within the exponential phase (phase 2; Figure 1 and Figure 2) of the amplification curve (signal intensity doubling in each cycle) before it gets into a phase with steady linear increase of the signal intensity (phase 3; Figure 1 and Figure 2).

Note: The adjustment is described for the linear view. For AriaMx™ make sure graph type “Linear” is selected. For Bio-Rad CFX™ ensure that the box “Log Scale” is unchecked.

Agilent AriaMx™ software

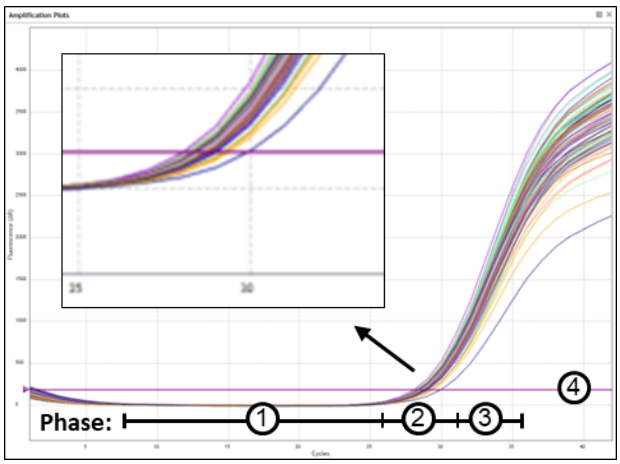

Figure 1. Exemplary amplification plot of a real-time PCR in linear scale (graph type "Linear"). 1) background; 2) exponential phase; 3) linear amplification phase; 4) threshold line.

Bio-Rad CFX™ software

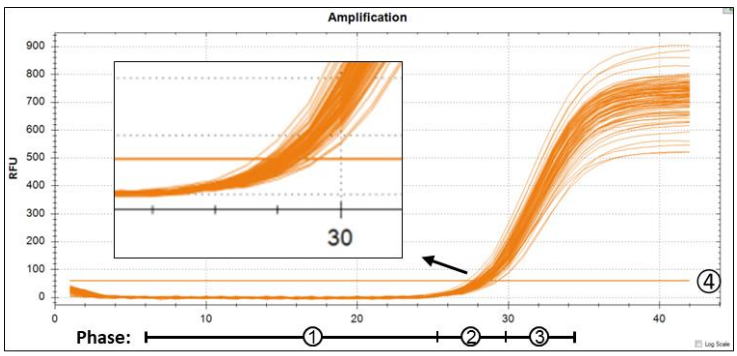

Figure 2. Exemplary amplification plot of a real-time PCR in linear scale (box "Log Scale" is unchecked). 1) background; 2) exponential amplification phase; 3) linear amplification phase; 4) threshold line.

The following tables provide an overview of the criteria to evaluate the run files:

Control evaluation

Control type	Cq HEX value	EPF HEX value	Cq FAM value	EPF FAM value	Overall results
Positive control VIR*Type* mutant (L452R)	22 ≤ Cq ≤ 31	n/a	25 ≤ Cq ≤ 34	n/a	Valid
	Cq < 22	n/a	Not relevant	n/a	Invalid*
	Cq > 31	n/a	Not relevant	n/a	Invalid*
	Not relevant	n/a	Cq < 25	n/a	Invalid*
	Not relevant	n/a	Cq > 34	n/a	Invalid*
Negative control (C^-)	No Cq	n/a	No Cq	n/a	Valid
	Cq ≤ 40	n/a	Not relevant	n/a	Invalid*
	Not relevant	n/a	Cq ≤ 40	n/a	Invalid*

EPF = Endpoint Fluorescence

**Check amplification curve for sigmoid amplification signals, software background calculation and threshold settings*

In case you included a negative extraction control in your analyses, scoring of single targets is performed as described for regular samples (see below).

Scoring of samples Agilent AriaMx™ and Bio-Rad CFX96 Touch™

Cq HEX™ value	EPF HEX™ value	Cq FAM™ value		EPF FAM™ value	Target specific results
Cq ≤ 40	n/a	No Cq		n/a	Positive (POS) for SARS-CoV-2 Negative (NEG) for L452R
No Cq	n/a	No Cq		n/a	Negative (NEG) for SARS-CoV-2 Negative (NEG) for L452R
Cq ≤ 40	n/a	Cq ≤ 40		n/a	Positive (POS) for SARS-CoV-2 Positive (POS) for L452R
No Cq	n/a	Cq ≤ 40		n/a	Invalid

Final result scoring for samples

	Detection Channel	
	HEX™	FAM™
SARS-CoV-2 RdRP	POS	POS
Variant carrying L452R single point mutation	NEG	POS
Mix of wild type and L452 variant	POS	POS

Discrimination of the virus variant with respect to the S-gene codons 452 is also possible for samples that have already been pretested positive for the presence of SARS-CoV-2 RNA and whose Cq values were scored positive as followed for VIR*Seek* SARS-CoV-2 Mplex Cq < 37 and for VIR*Seek* SARS-CoV-2 Ident 2 Cq < 36.

As environmental samples including wastewater tend to be of mixed nature that may contain different SARS-CoV-2 variants, one needs to consider asymmetric effects given by the nature of those mutant type assays. The respective SARS-CoV-2 variant containing L452R single point mutation is only detectable within a range of 10-100% variant in a mixed sample of SARS-CoV-2 wild type / variant mix.

4 REFERENCES

1. van Doremalen *et al.*, “Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1”, Correspondence, New England Journal of Medicine, 17.03.2020, DOI: 10.1056/NEJMc2004973
2. https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html#Interest
3. COVID-19 Weekly Epidemiological Update Data as received by WHO from national authorities, as of 25 April 2021, 10 am CET
4. Corman *et al.*, “Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR “, Eurosurveillance, Volume 25, Issue 3
5. World Health Organization, “Surface sampling of coronavirus disease (COVID-19): A practical “how to” protocol for health care and public health professionals “, Version: 1.1, February 2020, www.who.int
6. World Health Organization, “Laboratory testing for coronavirus disease (COVID-19) in suspected human cases”, Interim guidance 19.03.2020, www.who.int

5 PRODUCT WARRANTIES, SATISFACTION GUARANTEE

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6 PRODUCT USE LIMITATIONS

This kit is developed, designed, and sold for research purposes only. It is not to be used for diagnostic purposes or analysis of food and feed unless expressly cleared for that purpose by the competent regulatory authorities in the country of use. All due care and attention should be exercised in the handling of the materials described in this text.

7 IMPORTANT NOTES

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For technical assistance and more information please contact the Eurofins GeneScan Technologies GmbH Customer Service or your local distributor.

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