

VIRType SARS-CoV-2 (K417N)

TEST KIT FOR SARS-COV-2 VARIANT DETECTION CARRYING
K417N MUTATION USING RT-REAL-TIME PCR FROM FOOD &
ENVIRONMENTAL SURFACES AND WASTEWATER

Cat. No. 5728401501

For 48 RT-qPCR reactions

VIRSeek

Table of Contents

1	INTRODUCTION.....	2
1.1	Test Principle.....	3
1.2	Components of the Kit.....	4
1.3	Additional Equipment, Consumables and Reagents Required.....	4
1.4	Complementary Portfolio of the VIRSeek Solution	6
2	How to use this product.....	7
2.1	Important Notes.....	7
2.2	General and Safety Precautions	7
2.3	Working Guidelines	7
2.4	RNA Handling – Specific Working Guidelines	8
2.5	Cleaning Protocol.....	9
2.6	Waste disposal	9
2.7	RT-qPCR.....	9
2.7.1	Special Precautions during RT-qPCR Analysis	9
2.7.2	PCR Setup	10
3	Data Interpretation.....	12
3.1	Export of Raw Data	12
3.2	Evaluation of Results	13
4	References	15
5	PRODUCT WARRANTIES, SATISFACTION GUARANTEE.....	16
6	PRODUCT USE LIMITATIONS	16
7	IMPORTANT NOTES.....	16

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).

Most people infected with the COVID-19 virus will experience mild to moderate respiratory illness and recover without requiring special treatment. Older people and those with underlying medical conditions e.g. like cardiovascular disease, diabetes, and chronic respiratory disease are more likely to develop serious illness.

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.

Profound analysis of viral genomes, as a result of a rapid increase in COVID-19 cases especially in South-East England late 2020, led to the identification of new SARS-CoV-2 variants often carrying multiple spike protein mutations as well as mutations in other genomic regions. The spike protein is key for viral entry and for vaccine development. Therefore, specific identification of these variants is critical.

The VIRType SARS-CoV-2 (K417N) kit detects one single point mutation which result in the exchange of the amino acid Lysine to Asparagine in the spike protein at position 417. Mutations at position 417 is found in two variant lineages, however the exchange to an Asparagine is specific for South Africa lineage B.1.351 and can help the precise distinction of the different variant types of SARS-CoV-2.

The primer / probe combination of this RT-qPCR system is highly specific for the two SARS-CoV-2 lineage B 1.351 and does not cross-react with the S-gene region of SARS-CoV-2 wild type strain.

The VIRType SARS-CoV-2 (K417N) kit provides all reagents for the rapid detection of the South Africa lineage B 1.351 on food and environmental surfaces as well as wastewater via RT-qPCR. Single point mutation referring to the K→N amino acid exchange specific for South Africa variant is detected in the FAMTM, whilst respective wild type S-gene region is detected in the HEXTM channel.

The VIRType SARS-CoV-2 (K417N) kit is validated for use with the Agilent AriaMxTM, Bio-Rad CFX96 TouchTM and the CFX96 TouchTM 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 surfaces, such as described in the PathoSwab 50, followed by a suitable RNA extraction approach, e.g. *VIRSeek RNAExtractor* (see section 1.4), is required for these sample types. More specifically, we recommend the protocol provided by ISO 15216-2 using a sterile swab for sampling from food surfaces. For environmental surface sampling we recommend following the respective WHO guideline (see reference 3), before extracting the viral RNA.

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 e.g. *VIRSeek SARS-CoV-2 Mplex* or *VIRSeek SARS-CoV-2 Ident 2* (see section 1.4). The Mplex kit detects two targets on the N-gene sequence and primer/probes do not cross-react with SARS-CoV, MERS-CoV, or the seasonal human coronaviruses HKU1, OC43, NL63, 229E. The *VIRSeek SARS-CoV 2 Ident 2* specifically detects the RdRP-gene sequence.

In order to specifically detect SARS-CoV-2 variants from food or environmental surfaces as well as wastewater, the *VIRType SARS-CoV-2 (K417N)* kit can be applied as a confirmatory assay to detect mutation specific for the South Africa lineage B.1.351 using the same rapid thermal profile as the *VIRSeek SARS-CoV-2 Mplex* and the *VIRType SARS-CoV-2 Mplex (N501Y, A570D)*.

1.2 Components of the Kit



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

- 1x **Oligo Mix VIRType SARS-CoV-2 (K417N)**, vials with green white caps, contains primers /probes for K417N single point mutation as well as wild type K417 in S-Gene region, 159 µL, store at -20 °C ± 2 °C, do not freeze / thaw more than 3 times.
- 1x **Basic Mix VIRType SARS-CoV-2 (K417N)**, vials with white caps, 265 µL, store at -20 °C ± 2 °C, do not freeze / thaw more than 3 times.
- 1x **Positive Control VIRType SARS-CoV-2 HEX™ (K417)**, vial with red cap, 100 µL, store at -20 °C ± 2°C, do not freeze / thaw more than 3 times.
- 1x **Positive Control VIRType SARS-CoV-2 FAM™ (K417N)**, vial with red and white striped white cap, 100 µL, store at -20 °C ± 2°C, do not freeze / thaw more than 3 times.
- 1x **Negative Control VIRType SARS-CoV-2 (K417N)**, 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**

1.4 Complementary Portfolio of the VIRSeek Solution

- **VIRSeek 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.
- **VIRSeek 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.
- **VIRType SARS-COV-2 Mplex (N501Y, A570D)**, cat. no. 5728401301
 - RT-qPCR kit with 48 reactions for the detection of the N501Y and A570D single point mutations SARS-CoV-2 in food and environmental surface samples as well as in wastewater.
- **VIRType 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.

- **VIRSeek 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).
- **VIRSeek RNAExtractor**, cat. no. 5524400101
 - Kit for extraction of viral RNA via silica-coated magnetic beads.
- **VIRSeek RNAExtractor 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/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 immunocompromised 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 after usage.
- Store VIRType 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 VIRType Positive Control HEX™, VIRType Positive Control FAM™ or VIRType 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 VIRType SARS-CoV-2 (K417N) Assay

Designation	Volume of reaction mix	Addition of
Test samples	8 µL	12 µL of sample
Positive control (C ⁺) HEX TM (K417)	8 µL	12 µL of VIRType Positive Control HEX TM (K417)
Positive control (C ⁺) FAM TM (K417N)	8 µL	12 µL of VIRType Positive Control FAM TM (K417N)
Negative control (C ⁻)	8 µL	12 µL of VIRType Negative Control

Thermal Profile for AriaMxTM and Bio-Rad CFX96 TouchTM

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 TouchTM Standard and Deep Well use default ramp rate.

Probe / Detection System

VIRType SARS-CoV-2 (K417N)	S-gene variant	Dye
SARS-CoV-2 S-gene	K417N mutation	FAM TM
	K417 wildtype	HEX TM

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

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

Control evaluation

Control type	Cq HEX	Cq FAM	Overall results
Positive control VIRType HEX™(C+) (K417)	$25 \leq Cq \leq 34$	$Cq \geq 40$	Valid
	$25 \leq Cq \leq 34$	$Cq < 40$	Invalid*
	$Cq < 25$	Not relevant	Invalid*
	$Cq > 34$	Not relevant	Invalid*
Positive control VIRType FAM™(C+) (K417N)	$Cq \geq 40$	$25 \leq Cq \leq 34$	Valid
	$Cq < 40$	$25 \leq Cq \leq 34$	Invalid*
	Not relevant	$Cq < 25$	Invalid*
	Not relevant	$Cq > 34$	Invalid*
Negative control (C-)	$Cq \geq 40$	$Cq \geq 40$	Valid
	$Cq < 40$	Not relevant	Invalid*
	Not relevant	$Cq < 40$	Invalid*

*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

Target name	Cq result	Target specific results
SARS-CoV-2 S-gene	Cq ≤ 40	Positive (POS)
	No Cq	Negative (neg)
	Detection Channel	
	HEX™	FAM™
Wild type	POS	neg
Lineage B 1.351 – South Africa	neg	POS

Discrimination of virus variants with respect to the S-gene codon 417 is only possible for samples:

1. that have already been pretested positive for the presence of SARS-CoV-2 RNA (recommendation to use VIRSeek SARS-CoV-2 Mplex or VIRSeek SARS-CoV-2 Ident 2) and
2. whose Cq values in one or both of the S-gene channels is below Cq ≤ 40.

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. Corman *et al.*, "Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR ", Eurosurveillance, Volume 25, Issue 3
3. 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
4. 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

Eurofins GeneScan Technologies GmbH (“GeneScan”) warrants the products manufactured by it will be free of defects in materials and workmanship when used in accordance with the applicable instructions before the expiration date marked on the product packaging and when stored under the storage conditions recommended in the instructions and/or on the package. GeneScan makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.

GeneScan’s sole obligation with the respect to the foregoing warranties shall be, at its option, to either replace or to refund the purchase price of the product(s) or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies GeneScan promptly of any such defect. GeneScan shall not be liable for any direct, indirect or consequential damages resulting from economic loss or property damages sustained by buyer or any customer from the use of the product(s). A copy of Eurofins GeneScan Technologies GmbH terms and conditions can be obtained on request, and is also provided in our price lists.

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

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.



TECHNICAL SUPPORT SERVICE

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

Eurofins GeneScan Technologies GmbH

Engesser Str. 4

79108 Freiburg, Germany

Phone: + 49-(0)761-5038-200

Fax: + 49-(0)761-5038-111

kits@eurofins.com

© Eurofins GeneScan Technologies GmbH, all rights reserved.