VIR*Seek* SARS-CoV-2 Ident 2

TEST KIT FOR SARS-COV-2 IDENTIFICATION (RDRP-GENE TARGET) QUALITATIVE REAL-TIME RT-PCR FROM ENVIRONMENTAL SAMPLES AND FOOD SURFACES

Cat. No. 5728200705

For 48 real-time RT-PCR 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).

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 problems e.g. like cardiovascular disease, diabetes, and chronic respiratory disease are more likely to develop serious illness.

Main route of transmission is mainly from person to person via respiratory droplets from coughs and sneezes. Potential indirect route of transmission e.g. by touching surfaces is discussed, as data have been generated proofing a survival of SARS-CoV-2 on surfaces such as stainless steel for up to 72 hours (see 1.2.1). These findings render testing of environmental samples 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.

The VIR*Seek* SARS-CoV-2 Ident 2 kit provides all reagents for the rapid detection of the SARS-CoV-2 RdRP-gene and identification of SARS-CoV-2 in environmental samples and food surfaces via real-time RT-PCR. An adequate protocol for sampling of viral material, followed by a suitable RNA extraction approach is required for these sample types.

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

The kit is intended to be used by analytical laboratories for environmental samples as part of quality control / quality assurance testing, (e.g. virological monitoring of production processes) or food surface testing.

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

1.1 Test Principle

After sampling of viral particles from environmental samples or food surfaces and subsequent extraction of viral RNA the VIR*Seek* SARS-CoV-2 Ident 2 kit can be used for the detection of the SARS-Cov-2 RdRP-gene. The first step of a real-time RT-PCR is a reverse transcription (RT) of viral RNA to cDNA, which can then be amplified by real-time PCR. For the extraction of RNA we recommend the VIR*Seek* RNA*Extractor* kit (see section 1.4.1).

For sampling from food surfaces, we recommend the protocol provided by ISO 15216-2:2019 by using a sterile swab. For environmental surface sampling we recommend following the respective WHO guideline (see 1.2.2), before extracting the viral RNA. According to ISO 15216-2:2019 the processes and horizontal methods for detection of e.g. HAV and norovirus using real-time RT-PCR in food (surface) samples require the usage of a process control virus in order to verify and monitor the RNA extraction efficiency throughout the process. Although, no respective guideline for SARS-CoV-2 detection is available at the moment, we recommend verifying the efficiency of viral RNA extraction with the VIR*Seek* Murine Norovirus (MNV) Process Control kit (see section 1.4.1).

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. By means of specific primer nucleotide sequences of the SARS-CoV-2 RdRP-gene are amplified during PCR from isolated and reverse-transcribed total RNA. Primers do not cross-react with transcribed RNA (cDNA) from other common food-borne virus species, including norovirus genogroup I & II, hepatitis A & E virus, rotavirus, adenovirus or astrovirus.

1.2 References

1.2.1 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

1.2.2 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

1.3 Components of the Kit

For real-time RT-PCR: cat. no.5728200701

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

- 1x **OligoMix SARS-CoV-2 Ident 2**, vial with orange cap, contains primers / probes for IPC / SARS-CoV-2 RdRP-gene and IPC-RNA, 530 µL, store at -20 °C ± 2 °C, do not freeze / thaw more than 5 times.
- 1x **BasicMix*** **VIR*Seek***, vial with white cap, 265 µL, store at -20 °C ± 2 °C, do not freeze / thaw more than 5 times.
- 1x **Positive Control SARS-CoV-2**, vial with red cap, 50 µL, store at -20 °C ± 2 °C, do not freeze / thaw more than 5 times.
- 1x **Negative Control**, vial with transparent cap, 500 µL, store at -20 °C ± 2 °C.

1.4 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.
- 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 sets. For combining different kits from the SARS-CoV-2 solution, additional filters are required. Please refer to the respective manuals for further information.
- Bio-Rad CFX96 Touch™ (CFX Manager™ Software / CFX Maestro™ Software).
- Bio-Rad CFX96 Touch™ Deep Well (CFX Manager™ Software / CFX Maestro™ Software).

Consumables:

- **RNase-free water** (molecular biology grade).
- **DNA- / 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).
- **RNase-free pipette tips** need to be compatible with pipettes used.
- **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.

1.4.1 VIR*Seek* SARS-CoV-2 Solution

- **VIR*Seek* SARS-CoV-2 Screen**, cat. no. 5728200601
  - Real-time RT-PCR kit with 96 reactions for rapid screening for SARS-CoV-2 E-gene sequence in environmental and food surface samples.
- **VIR*Seek* Murine Norovirus (MNV) Process Control**, cat. no. 5728200401
  - Murine norovirus spiking material (1 mL) and real-time RT-PCR kit with 48 reactions for rapid detection of murine norovirus (MNV) process control virus.
- **VIR*Seek* RNA*Extractor***, cat. no. 5524400101
  - Kit for extraction of viral RNA via silica-coated magnetic beads.

2 HOW TO USE THIS PRODUCT

2.1 Important Notes

- Store all reagents as indicated in section 1.3.
- During PCR 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.

2.2 General and Safety Precautions

- All samples should be handled with caution 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.
- The VIR*Seek* kit contains glycerol and propane-1,2-diol which may cause mild skin irritation.For more information, please refer to the VIR*Seek* kit safety information.

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 (s. 1.2.2) 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 tips and consumables which are guaranteed to be RNase-free.
- Control high risk areas for DNA / amplicon contamination on a regular basis (swabs / PCR analysis).
- Clean the real-time RT-PCR 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.
- Avoid breathing on samples or working area: Wear a disposable face mask or protect the designated RNase-free working area with a screen.
- Always thaw RNA on a cooling block and store RNA at -20 °C or below.
- Handle real-time RT-PCR 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 VIR*Seek* kit components for real-time RT-PCR 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 real-time RT-PCR working area is cleaned as follows:

Cleaning steps	Cleaning protocol
1.	Decontaminate surfaces with Roti® Nucleic Acid-free* or 1 % HCl 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 the safety information.

2.7 Before you Begin

Store the cooling block for real-time RT-PCR at -20 °C overnight.

For RNA extraction use suitable RNA extraction kits, for optimal performance we recommend to use Eurofins GeneScan Technologies' VIR*Seek* RNA*Extractor* kit (see section 1.4.1).

2.8 Real-Time RT-PCR

2.8.1 Special Precautions during Real-Time RT-PCR 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.

2.8.2 PCR Setup

Keep all components (OligoMix and BasicMix) permanently on ice/cooling block during
PCR setup.
Prepare final reaction mix fresh each time and immediately before starting the real-time RT-PCR run.

Calculate required number of reactions and pipette all components (OligoMix and BasicMix) together and mix for the final reaction mix. The final real-time RT-PCR reaction mix is prepared with an additional 10 % volume.

Frequent freezing and thawing might cause inactivation of the reagents (see 1.3).

Components of final reaction mix	Amount per reaction	e.g. for 10 real-time RT-PCR reactions (+ 10 %)
BasicMix	5 µL	55 µL
OligoMix	10 µL	110 µL
Total volume	15 µL	165 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 15 µL of final reaction mix to each test well.
3. Add 5 µL Positive Control SARS-CoV-2, Negative Control, negative extraction control, negative sampling control, and negative sampling device control sample to the corresponding wells.
4. Add 5 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 real-time RT-PCR 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*Seek* SARS-CoV-2 Ident 2 Assay

Designation	Volume of reaction mix	Addition of
Test samples	15 µL	5 µL of sample
Positive control (C^+)	15 µL	5 µL of Positive Control SARS-CoV-2
Negative control (C^-)	15 µL	5 µL of Negative Control
Negative extraction control (E^-)	15 µL	5 µL of negative extraction control sample
Negative sampling control (S^-)	15 µL	5 µL of negative sampling control sample
Negative sampling device control (SD^-)	15 µL	5 µL of negative sampling device control sample

Plate Setup

The following PCR plate setup is recommended, if samples are analysed for SARS-CoV-2 RdRP-gene and the process control virus. The controls correspond to the controls recommended by ISO 15216-2:2019 and the respective WHO guideline (see 1.2.2).

	1	2	3	4	5	6	7	8	9	10	11	12
A	C^+	S1-1[2]					(E^-)[1]	(S1)[1]				
B	C^-	S1-2[2]					(C^-)[1]	(Sn)[1]				
C	E^-	Sn-1[2]					(PC^+)[1]					
D	S^-*	Sn-2[2]					(PC 10^{-1})[1]					
E	SD^-*						(PC 10^{-2})[1]					
F							(PC 10^{-3})[1]					
G												
H												

[1] *Run with process control virus real-time RT-PCR Assay (e.g. VIRSeek Murine Norovirus (MNV) Process Control, refer to section 1.4.1)*

[2] *ISO 15216-2:2019 recommends testing samples in PCR duplicates*

** The respective WHO guideline (see 1.2.2) recommends including negative swab and swabbing device samples.*

- C^+: positive control (of the target of interest)
- C^-: negative control
- E^-: negative extraction control
- S^-: negative sampling control
- SD^-: negative sampling device control
- S1-1 – Sn-2: test samples (if run in duplicates), single test reactions are feasible
- (PC+): process control
- (PC (10-1/10-2/10-3)): process control standard (10-1 / 10-2 / 10-3 dilution of RNA extraction from process control sample)

Thermal Profile

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	3 sec at 95 °C	30 sec at 58 °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*Seek* SARS-CoV-2 Ident 2	Fluorophore (Dye)
SARS-CoV-2 RdRP-gene	FAM™
IPC	Cy5™

3 DATA INTERPRETATION

Data is analysed by using the appropriate software provided by the cycler manufacturer.
For the validated cyclers we recommend the following settings:

Real-time RT-PCR Thermocycler	Threshold	Baseline
Agilent AriaMX™	Auto[1)]	Adaptive
Bio-Rad CFX96 Touch™	Auto	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	SARS-CoV-2 RdRP-gene	IPC	Overall results
Positive control (C^+)	25 ≤ Cq ≤ 34	Not relevant	Valid
	Cq < 25		Invalid*
	Cq > 34		Invalid*
Negative control (C^-)	Cq > 39	Cq ≤ 37	Valid
	Cq > 39	Cq > 37	Invalid*
	Cq ≤ 39	Not relevant	Invalid*

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

Scoring of samples

Target name	**Cq result**	**Target specific results**
SARS-CoV-2 RdRP-gene	Cq ≤ 39	Positive
	Cq > 39	Negative
	No Cq	Negative
IPC	Cq (C^-) -3 ≤ Cq Sample ≤ Cq (C^-) +3	Valid
	Cq Sample < Cq (C^-) -3	Unexpected result. Check amplification curve for sigmoid amplification signals, software background calculation and threshold settings.
	Cq Sample > Cq (C^-) +3	Sample inhibited
	No Cq	Sample inhibited

Result interpretation for sample duplicates

Replicate 1	Replicate 2	Final Result
Positive for SARS-CoV-2 RdRP-gene	Positive for SARS-CoV-2 RdRP-gene	Positive for SARS-CoV-2 RdRP-gene
Positive for SARS-CoV-2 RdRP-gene	Negative for SARS-CoV-2 RdRP-gene	Positive for SARS-CoV-2 RdRP-gene
Negative for SARS-CoV-2 RdRP-gene	Positive for SARS-CoV-2 RdRP-gene	Positive for SARS-CoV-2 RdRP-gene
Negative for SARS-CoV-2 RdRP-gene	Negative for SARS-CoV-2 RdRP-gene	Negative for SARS-CoV-2 RdRP-gene

Final result interpretation for qualitative SARS-CoV-2 RdRP-gene real-time RT-PCR assay (including process control virus)

Preliminary sample result	IPC	Process control virus	Final results	Warning / measure
Positive for SARS-CoV-2 RdRP-gene	Not relevant	Not relevant	Positive for SARS-CoV-2 RdRP-gene	
Negative for SARS-CoV-2 RdRP-gene	Not inhibited	Valid	Negative for SARS-CoV-2 RdRP-gene	
Negative for SARS-CoV-2 RdRP-gene	Inhibited	Valid	Sample is inhibited	Test 1:10 dilution of RNA extract of undiluted sample; see also ISO 15126-2: 2019. As option: test also 1:5 dilution of RNA extract of undiluted sample. If 1:10 dilution is still inhibited, repeat RNA extraction of the sample. For using the option: If both dilutions (1:10 and 1:5) are still inhibited, repeat RNA extraction of the sample.

Preliminary sample result	IPC	Process control virus	Final results	Warning / measure
Negative for SARS-CoV-2 RdRP-gene	Inhibited	Invalid	Extraction efficiency of process control virus too low and sample is inhibited	Test 1:10 dilution of the process control virus-sample and 1:10 dilution of RNA extract of undiluted sample. As option: test also 1:5 dilution of RNA extract of undiluted sample. If 1:10 dilution is still inhibited, repeat RNA extraction of the sample. For using the option: If both dilutions (1:10 and 1:5) are still inhibited, repeat RNA extraction of the sample. If the diluted process control virus-sample is still invalid, repeat virus extraction.
Negative for SARS-CoV-2 RdRP-gene	Not inhibited	Invalid	Extraction efficiency of process control virus too low and/or sample inhibited	Test 1:10 of sample of process control virus. If process control virus is still invalid, repeat virus extraction.

Please note: Duplicates should be tested according to ISO 15216. The interpretation of results of duplicated samples is described in Table "Result interpretation for sample duplicates".

Final result interpretation for qualitative SARS-CoV-2 RdRP-gene real-time RT-PCR assay (without process control virus)

Preliminary sample result	IPC	Final results	Warning / measure
Positive for SARS-CoV-2 RdRP-gene	Not relevant	Positive for SARS-CoV-2 RdRP-gene	
Negative for SARS-CoV-2 RdRP-gene	Not inhibited	Negative for SARS-CoV-2 RdRP-gene	
Negative for SARS-CoV-2 RdRP-gene	Inhibited	Sample is inhibited	Test 1:10 dilution of RNA extract of undiluted sample; see also ISO 15126-2: 2019. As option: test also 1:5 dilution of RNA extract of undiluted sample. If 1:10 dilution is still inhibited, repeat RNA extraction of the sample. For using the option: If both dilutions (1:10 and 1:5) are still inhibited, repeat RNA extraction of the sample.

4 PRODUCT WARRANTIES, SATISFACTION GUARANTEE

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5 PRODUCT USE LIMITATIONS

This kit is developed, designed, and sold for research purposes only. It is not to be used for diagnostic purposes 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.

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TECHNICAL SUPPORT SERVICE

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