ViroBOAR RT-PCR Kit (SARS-CoV-2)

User Manual

For *in-vitro* diagnostic use only

For use with Roche LightCycler 480 II Instrument

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100/1000 REF 6100-ViroBO -80°C -20°C

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1. Introduction

End of 2019, a novel respiratory disease emerged in the city of 5.1.2a 5.1.2a, and soon spread rapidly within the country and worldwide. The causative agent was identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 (2019-nCoV), like the closely related SARS coronavirus (SARS-CoV), belongs to the genus Betacoronavirus within the family of coronaviruses. The zoonotic reservoir of the virus appears to be bats.

Coronaviruses are enveloped, positive single-stranded large RNA viruses that infect humans, but also a wide range of animals. The common human coronaviruses NL63, 229E, OC43 and HKU1 are widespread especially throughout the winter months. They are responsible for up to one third of all acute respiratory diseases, typically with mild symptoms (common cold). More than 80 % of the adult population have antibodies against human coronaviruses. The immunity from previous infections lasts only for a short period of time. Therefore, reinfections with the same pathogen are possible just after one year.

SARS-CoV-2 is predominantly transmitted by droplet infection via coughing or sneezing and through close contact with infected patients. In theory, smear infection and infection through the conjunctiva of the eyes are also possible. The incubation period is in the median 5–6 days (and up to 14 days maximum).

The clinical manifestations of SARS-CoV-2-related COVID-19 disease include fever, cough, respiratory problems and fatigue. In most patients the infection manifests with symptoms of a mild febrile illness with irregular lung infiltrates.

The initial clinical sign of COVID-19 which allowed case detection was pneumonia. But it turned out that the course of the disease is non-specific and varies widely, from asymptomatic courses to severe pneumonia with lung failure and death. However, based on current knowledge, around 80 % of the illnesses are mild to moderate.

Although severe courses of the disease also occur in younger patients and people without previous illness, the following groups of people have an increased risk of serious forms of the disease: elderly people (with a steadily increasing risk from around 50-60 years of age), smokers and people with certain diseases of the cardiovascular system or the lungs, patients with chronic liver diseases, diabetes mellitus, cancer, or patients with a weakened immune system (e.g. due to immune deficiencies or by taking drugs that suppress the immune system).

Currently, there is no specific treatment or vaccine available against SARS-CoV-2 infection.

Species	Disease	Symptoms e.g.	Transmission route
SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2)	COVID-19	the course of the disease is unspecific, diverse and varies greatly, from asymptomatic courses to severe pneumonia with lung failure and death	primary mode of transmission: droplet infection; smear infections and infections via the conjunctiva of the eyes are theoretically possible

The presence of pathogen or infection may be identified by

- Nucleic acid testing (NAT): e.g. RT-PCR
- Serology: detection of antibodies by e.g. ELISA

2. Intended Use

The ViroBOAR RT-PCR Kit is used for the qualitative detection of SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) genomic RNA extracted from human respiratory specimen (e.g. nasal wash/swab, nasopharyngeal wash/swab and oropharyngeal swab as described in WHO interim guidance "*Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases*") by RT- PCR method. The ViroBOAR RT-PCR Kit is intended for use by trained laboratory personnel only.

3. Principle of the RT-PCR Assay

The kit contains a specific ready-to-use system for the detection of Novel Coronavirus (2019-nCoV) by Reverse Transcription Polymerase Chain Reaction (RT-PCR) in the real-time PCR system. The reaction is done in one tube two step real-time RT-PCR. The first step is a reverse transcription (RT), during which the virus RNA is transcribed into cDNA. Afterwards, a thermostable DNA polymerase is used to amplify the specific gene fragments by means of polymerase chain reaction (PCR). Fluorescence is emitted and measured by the real-time systems´ optical unit during PCR. The detection of amplified virus DNA fragment is performed in fluorimeter channel FAM (465-510 nm > detection of N1-gene), HEX/VIC/Yellow555 (533-580 nm > detection of IPC) and Cy5/Cy5.5 (618-660 nm > detection of E-gene) with Black Hole Quencher 1 (BHQ1) and Black Hole Quencher 2 (BHQ2).

The principle of the real-time detection is based on the fluorogenic 5' nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (Cp) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in real-time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

4. Material Provided

Component Nr.	Kit Components	Presentation (100 rxns)	Presentation (1000 rxn)
1	2x qPCR Mix	1 vial, 800 µl	5 vials, 1600 µl
2	Oligo/IPC Mix	1 vial, 50 µl	1 vial, 500 µl
3	20x Rtase	1 vial, 90 µl	1 vial, 900 µl
4	ddH_2O	1 vial, 70 µl	1 vial, 700 µl
5	pos Con (RNA), 250 copies/µl	1 vial, 100 µl	1 vial, 1000 µl

5. Stability and Storage

The ViroBOAR RT- PCR Kit is shipped on dry ice and all components should arrive frozen.

- All components have to be stored at -20 °C upon arrival.
- Storage at +4°C is not recommended for longer than 3 hours
- More than one Repeated freeze thaw cycles of reagents should be avoided, since this might affect the performance of the kit. Reagents should be frozen in aliquots if they are used intermittently.
- Keep unfrozen storage (e.g. storage on ice) as short as possible.
- Keep the kit components in the freezer, until you are ready to use it.
- Protect the Oligo/IPC Mix (component 2) from light.

6. Additionally Required Materials and Devices but not provided

- Biological cabinet/Laminar Airflow
- Vortex mixer
- Cryo-container
- Sterile filter tips for micro pipets
- Disposable gloves, powderless
- Refrigerator and freezer
- Roche LightCycler 480 II Instrument
- Pipets (0.5µl – 1000µl)
- Sterile microtubes
- Biohazard waste container
- Tube racks
- Desktop microcentrifuge for "Eppendorf" type tubes (RCF max. 16,000 x g)
- Extraction device

7. Sample Collection and Preparation

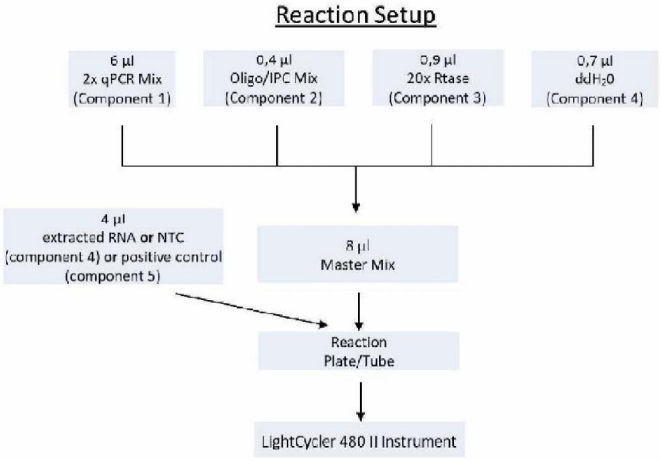
- Regarding sample collection and shipment please refer to the WHO interim guidance "*Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases*".
- Extracted RNA from human respiratory specimen types is the starting material for the ViroBOAR RT-PCR Kit. The quality of the extracted RNA has a crucial effect on the performance of the entire RT-PCR test system. Make sure that the nucleic acid extraction method is compatible with real-time PCR technology.
- For nucleic acid extraction a method suitable for extracting virus RNA from human respiratory specimen should be used.

- Since ethanol is a strong real-time PCR inhibitor, it is necessary to completely eliminate it prior to the elution of the nucleic acid during extraction. If using spin columns with washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step of 10 min at approximately 17,000 x g (~ 13,000 rpm) before eluting the RNA. For this additional centrifugation step, use a new collection tube.

8. Assay Procedure

8.1 Reaction Setup

- Please read the instructions for use carefully before performing the assay. Reliability of results depends on following strictly the instructions for use.
- Before use make sure that all samples and reagents are thawed completely, mixed by up and down pipetting or vortexing and centrifuged briefly.
- Prepare quickly the Reaction Mix on ice or in the cooling block.
- It is highly recommended to pipet samples and controls in triplicates.
- Pipette kit components slowly and carefully and use pipette tips suitable for pipetting viscous liquids.
- The use of ddH_2O (component 4) as no template control (NTC) is highly recommended.
- Define the positions of the wells on the plate for samples and controls (positive control or NTC).
- Multiply the volumes of 2xqPCR Mix (component 1), Oligo/IPC Mix (component 2), 20xRTase (component 3) and ddH_2O (component 4) per reaction with the number of samples, which includes the number of controls, standards, and samples prepared. ddH_2O (kit component 4) is set into the RT-PCR as no template control. Artificial RNA is used as positive control (component 5). For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely and then spin down briefly with a centrifuge.
- Pipet 8µl Master Mix with micropipets of sterile filter tips to each of the real-time PCR reaction plates/tubes. Separately add 4µl template (nucleic acid extracted from negative control and specimen, positive control RNA with no extraction) to different reaction plates/tubes. Immediately close the plates/tubes to avoid contamination.
- Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
- Perform the following protocol in the instrument of Roche LightCycler 480 II instrument.



8.2 Programming the Roche LightCycler 480 II Instrument

Regarding setup and programming of Roche LightCycler 480 II instrument, please use the corresponding manual provided by the manufacturer.

RT-PCR Run Settings:

Step	°C	Time	No. of Cycles
Reverse Transcription	45	10 min	1
Polymerase activation	95	2 min	1
Amplification	95	5 sec	50
	60	30 sec	

Before starting the test run, please check the settings for cycles, temperature and time.

Fluorescent Detectors/Dyes:

Detection	Gen	Dye	Quencher	Detection channel (Excitation/Emission)
SARS-CoV-2	2019nCoV_N1	FAM	BHQ1	465nm/510nm
SARS-CoV-2	2019nCoV_E	Atto647N	BHQ2	618nm/660nm
IPC	HPV	HEX	BHQ1	533nm/580nm

9. Data Analysis and Interpretation

Data analysis should be performed with the software of the Roche LightCycler 480 II instrument according to manufacturer's instructions

The following sample results are possible:

2019nCoV_N1	2019nCoV_E	positive Control (1000 copies/RT-PCR), mean values of replicates	IPC (30,000 copies/RT-PCR)	Report
Cp < 38	Cp < 38	Cp 26.5-32.5 (N1) Cp 24.0-2.0 (E)	Cp > 20 or sigmoid curve	Positive 2019-nCoV
Cp < 38	-	Cp 26.5-32.5 (N1) Cp 24.0-2.0 (E)	Cp > 20 or sigmoid curve	ambiguous
-	-	Cp 26.5-32.5 (N1) Cp 24.0-2.0 (E)	Cp > 20 or sigmoid curve	Negative 2019-nCoV
Cp < 38	Cp < 38	Cp 26.5-32.5 (N1) Cp 24.0-2.0 (E)	Cp > 30	Positive 2019-nCoV
Cp < 38	-	Cp 26.5-32.5 (N1) Cp 24.0-2.0 (E)	Cp > 30	ambiguous
-	-	Cp 26.5-32.5 (N1) Cp 24.0-2.0 (E)	Cp > 30	Negative 2019-nCoV
Cp < 38	Cp < 38	Cp 26.5-32.5 (N1) Cp 24.0-2.0 (E)	-	Positive 2019-nCoV
Cp < 38	-	Cp 26.5-32.5 (N1) Cp 24.0-2.0 (E)	-	ambiguous
-	-	Cp 26.5-32.5 (N1) Cp 24.0-2.0 (E)	-	Negative 2019-nCoV

The Cp ranges displayed for the positive control and IPC are fixed acceptance ranges.
In case of ambiguous results a diagnostic statement must not be made. The RT-PCR run should be repeated or a new sample must be analyzed.
Diagnosis of an infectious disease should not be established only on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as other laboratory diagnostics.

10. Specific Performance Characteristics

The determinations of the specific performance characteristics were determined with the Roche LightCycler 480 II.

Other commercially available SARS-Cov2 kits were used as reference method for the evaluation of performance characteristics.

To establish performance characteristics, RNA extraction was performed using the by using the *PurePrep Pathogens Kit* from Molg3n (Utrecht, Netherlands; Art.No.OE00290096) on KingFisher Flex instruments (ThermoFisher Scientific).

Use of other real-time PCR instruments other than the Roche LightCycler 480 II or RNA extraction methods other than the PurePrep Pathogens Kit must be validated by the user.

Validation Parameter	Sample	Results
Specificity	24 positive and 48 negative samples	100%
Sensitivity	24 positive and 48 negative samples	96%
Precision (Repeatability, Intermediate Precision)	10 positive samples	SD <0,5 Cp (repeatability); SD < 1,0 Cp (intermediate precision)
LOD	Dilution series of RNA positiv control (calibrated with digital PCR)	1 copy RNA/RT-PCR
Accuracy	12 positive and 48 negative samples	98,7%

Interference-effects can be detected by monitoring and analysis of the IPC signals (HEX/VIC/Yellow555 channel). The acceptance range of the IPC is shown in paragraph 9.

11. Quality Control

In accordance with Eurofins Genomics Europe Synthesis GmbH ISO-certified Quality Management System, each lot of the ViroBOAR RT-PCR Kit has been tested against predetermined specifications to ensure consistent product quality. A certificate of Analysis is provided with the kit on demand.

12. Trademarks and Disclaimers

LightCycler® 480 Instrument II (Roche)

KingFisher™ Flex Purification System (ThermoFisher Scientific)

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

13. Precautions and Warnings

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed.
- The use of the test kit with other analyses than the ones mentioned under "10. Specific Performance Characteristics" has to be validated.
- Any deviation from the test procedure as well as any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons.
- Only for *in-vitro* diagnostic use.
- Do not interchange reagents of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Wear disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Always use DNase/RNase-free disposable reaction tubes and pipette tips with aerosol barriers.

- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.
- In order to avoid contamination of working space with nucleic acids, reaction tubes/plates should not be opened after amplification.
- RT-PCR is highly sensitive to nucleic acid contamination. Therefore, positive/potentially positive material needs to be stored separate from all other components of the kit.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- This assay must not be used on the specimen directly.
- Prior to using this assay, the nucleic acid has to be extracted with suitable extraction methods from the original specimen.
- The result of this RT-PCR kit may be influenced by potential mutations in the genome of the pathogen if they are located in the primer / probe binding region. Underestimation and/or failure to detect the pathogen may occur.
- PCR inhibitors may also elicit underestimation, false negative results or invalid runs. Therefore, only use nucleic acids extraction kits, which remove PCR inhibitors and which are dedicated for downstream PCR processes.
- The RT-PCR is only designed for qualified personnel who are familiar with good laboratory practice and trained in Real Time-PCR technology.
- Avoid repeated thawing and freezing of reagents as this may reduce the sensitivity of the test.
- Avoid unnecessary light exposure from Oligo/IPC Mix (component 2)

14. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

15. Ordering Information

Prod. No.:	6100-ViroBO	ViroBOAR RT- PCR Kit

16. Bibliography

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SYMBOLS KEY

	Manufactured by
	Contains sufficient for "n" tests
	Protect from Light
	Expiration Date
	Storage Temperature
	Consult Instructions for Use
LOT	Lot Number
REF	Catalogue Number
CE	CE Mark

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