Eurofins Genomics Europe Synthesis GmbH

Revision No.: EGE211029
Issue Date: Oct 29th, 2021

ViroBOAR 4.0 RT-PCR Kit (SARS-CoV-2)

User Manual

REF 6000-ViroBO4.0

Σ 100/1000 80°C -20°C

CE IVD **For *in-vitro* diagnostic use only**

For use with Roche LightCycler 480 II Instrument

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1. Intended Use

The ViroBOAR 4.0 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. pharynx gargle lavage, 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 4.0 RT-PCR Kit is intended for use by trained laboratory personnel only.

2. Principle of Real-Time PCR

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 = crossing point) 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.

3. Product Description

On January 11, 2020, Chinese health authorities preliminarily identified more than 40 human infections with a novel coronavirus in an outbreak of pneumonia under investigation in Wuhan City, Hubei Province, China. The Chinese authorities identified a new type of coronavirus (novel coronavirus, named as SARS-CoV-2 virus), which was isolated on January 7, 2020.
Coronaviruses are a large family of viruses, some causing illness in human and others circulating among animals such as camels, cats and bats. 2019-nCoV is a novel coronavirus. Primer and probe sequences for this kit for the SARS-CoV E gene assay are taken from the WHO recommendations (https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-detection-of-sars-cov-2-institut-pasteur-paris.pdf?sfvrsn=3662fcb6_2) and for 2019-nCoV N gene assay as well as for the RNase P assay are taken from the CDC recommendations (DOI: https://doi.org/10.3201/eid2608.201246 and https://www.cdc.gov/coronavirus/2019-ncov/downloads/lab/multiplex-primers-probes-printer.pdf).
The kit contains a specific ready-to-use system for the detection of SARS-CoV-2 (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 during PCR and measured by the real time systems´ optical unit. The detection of amplified virus DNA fragment is performed in fluorimeter channel FAM (465-510 nm for the detection of N1 gene for Roche LightCycler), Cy5/Cy5.5/ATTO647N (618-660 nm for the detection of E gene for Roche LightCycler) and HEX/VIC/Yellow555 (533-580 nm for the detection of internal positive control for Roche LightCycler) and with Black Hole Quencher 1 (BHQ1) and Black Hole Quencher 2 (BHQ2).

4. Kit Contents

Component Nr.	Kit Components	Presentation (100 rxns)	Presentation (1000 rxn)
1	2x qPCR Mix	1 vial; 700 µl	1 vial; 7.0 ml
2	Oligo Mix	1 vial; 50 µl	1 via; 500 µl
3	20x Rtase	1 vial; 100 µl	1 vial; 1.0 ml
4	ddH20	1 vial; 80 µl	1 vial; 800 µl
5	pos Con (RNA), 250 copies/µl	1 vial; 100 µl	1 vial; 1.0 ml

Limit of detection: 1 copy/RT-PCR;
Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction method and other factors. If you use the RNA extraction kits recommended, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.
Diagnostic specificity: 100 %
Diagnostic sensitivity: 96 %
Clinical accuracy: 98.7 %

5. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended for longer than 3 hours
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (> 1x) should be avoided as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.

6. Materials and Devices required but Not Supplied with the Kit

- 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
- Real time PCR reaction tubes/plates
- Pipets (0.5µl – 1000µl)
- Sterile microtubes
- Biohazard waste container
- Tube racks
- Desktop centrifuge
- Viral RNA extraction kit

7. Warnings and Precautions

- Carefully read this instruction before starting the procedure.
- This assay needs to be carried out by trained laboratory personnel only.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- Do not use the kit beyond its expiration date.
- Avoid repeated thawing and freezing of reagents as this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction Mix on ice or in the cooling block.
- Avoid unnecessary light exposure from Oligo Mix (Component2)
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working areas.
- Use always sterile pipette tips with filters.
- Wear disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Wear separate coats and gloves in each area.
- Do not pipette by mouth. Do not eat, drink or smoke in laboratory.
- Avoid aerosols

8. Sample Collection, Storage and Transport

- Collect samples in sterile tubes
- Specimens can be extracted immediately or frozen at -20°C to -80°C
- Transportation of clinical specimens must comply with local regulations for the transport of potentially infectious agents

9. Procedure

9.1 RNA-Extraction

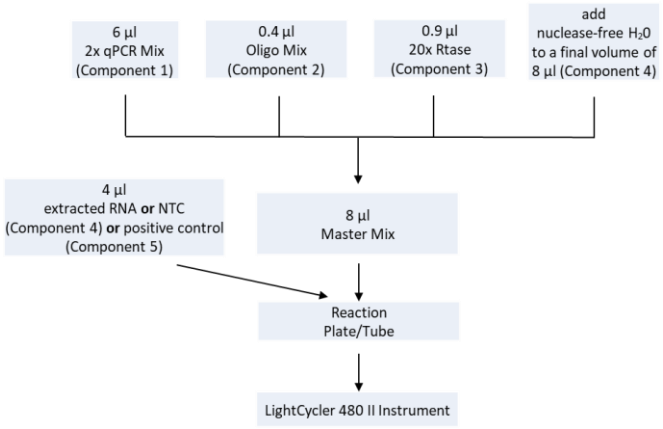
During kit development and validation, the PurePrep Pathogens Kit from Molgen and the GSD NovaPrime® RNA Extraction AE1 kit from Eurofins GeneScan Technologies was used on the Kingfisher Flex instrument (Thermofisher Scientific) *It is noted that the negative control should be nucleic acid extracted with the same protocol for specimens. The positive control doesn't need to be nucleic acid extracted.*

9.2 Internal Positive Control (IPC)

As an internal positive control (IPC), a section of human RNase P is amplified in parallel. This control indicates that intensive contact of the collection swab or gargling fluid with human mucosa has occurred. It also indicates that RNA extraction and qPCR were successful. The IPC is intended to give signals in the HEX channel. Results from accelerated stability tests indicate, that the RNase P assay might show an increase of variability after 1 month of storage at -20°C, which had no impact on detectability and data validity though.

9.3 RT-PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



1) Multiply the volumes of 2xqPCR Mix (Component 1), Oligo Mix (Component2), 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 (Component 4) can be set into the RT-PCR as no template control. Artificial RNA is used as positive control (Component 5). Adding an additional 10 % volume to the mastermix is recommended in order to balance out the pipetting loss. Mix completely and then spin down briefly with a centrifuge.
2) Pipette 8µl Master Mix with micropipettes 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 with no extraction) to different reaction plates/tubes. Immediately close the plates/tubes to avoid contamination.
3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
4) Perform the following protocol of the **LightCycler 480 II** instrument**:**

Step	°C	Time	No. of Cycles
Reverse Transcription	45	10 min	1
Polymerase activation	95	2 min	1
Amplification	95	5 sec	42
	60	30 sec	

10. PCR Control

Negative Control (no template PCR control) and Positive Control must be performed correctly; otherwise the sample results are invalid.

11. Quality Control

Negative Control (non target PCR control) and Positive Control must be performed correctly; otherwise the sample results are invalid.

Note: When using a Roche LightCycler as qPCR instrument non template controls show in very rare cases late signals (Cp ≥ 35) in the channel for detection of the Corona E gene.

12. Data Analysis and Interpretation

Data analysis should be performed with the software of the Roche LightCycler 480 II instrument according to manufacturer's instructions. In order to exclude false negative results due to possible matrix effects, the limit for the evaluation of a sample as positive is 35 cycles for qPCR of the N1 gene and 33 cycles for qPCR of the E gene using Roche LightCycler.

The Cp ranges displayed for the positive control are fixed acceptance ranges. It is recommended, that user confirm these values during internal kit verification.

In cases where clear signals occur only in one of the two analyzed target genes below the defined limit or in both target genes above the defined limits, no clear evaluation can be derived. Thus, in these cases a retest is recommended.

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.

2019nCoV_N	2019nCoV_E	positive Control (1000 copies/RT-PCR), mean values of replicates	IPC (RNase P)	Report
Cp ≤ 35	Cp ≤ 33	Cp 20.0-32.5 (N) Cp 20.0-29.0 (E)	Amplicon detected	Positive 2019-nCoV
Cp ≤ 35	Cp > 33	Cp 20.0-32.5 (N) Cp 20.0-29.0 (E)	Amplicon detected	Positive 2019-nCoV
Cp > 35	Cp ≤ 33	Cp 20.0-32.5 (N) Cp 20.0-29.0 (E)	Amplicon detected	Positive 2019-nCoV
Cp ≤ 35	-	Cp 20.0-32.5 (N) Cp 20.0-29.0 (E)	Amplicon detected	Retest*
-	Cp ≤ 33	Cp 20.0-32.5 (N) Cp 20.0-29.0 (E)	Amplicon detected	Retest*
Cp > 35	Cp > 33	Cp 20.0-32.5 (N) Cp 20.0-29.0 (E)	Amplicon detected	Retest*
Cp > 35	-	Cp 20.0-32.5 (N) Cp 20.0-29.0 (E)	Amplicon detected	Negative 2019-nCoV
-	Cp > 33	Cp 20.0-32.5 (N) Cp 20.0-29.0 (E)	Amplicon detected	Negative 2019-nCoV
-	-	Cp 20.0-32.5 (N) Cp 20.0-29.0 (E)	Amplicon detected	Negative 2019-nCoV
Cp ≤ 35	Cp ≤ 33	Cp 20.0-32.5 (N) Cp 20.0-29.0 (E)	No amplicon detected	Positive 2019-nCoV
Cp ≤ 35	Cp > 33	Cp 20.0-32.5 (N) Cp 20.0-29.0 (E)	No amplicon detected	Positive 2019-nCoV
Cp > 35	Cp ≤ 33	Cp 20.0-32.5 (N) Cp 20.0-29.0 (E)	No amplicon detected	Positive 2019-nCoV
Cp ≤ 35	-	Cp 20.0-32.5 (N) Cp 20.0-29.0 (E)	No amplicon detected	Invalid
-	Cp ≤ 33	Cp 20.0-32.5 (N) Cp 20.0-29.0 (E)	No amplicon detected	Invalid
Cp > 35	Cp > 33	Cp 20.0-32.5 (N) Cp 20.0-29.0 (E)	No amplicon detected	Invalid
Cp > 35	-	Cp 20.0-32.5 (N) Cp 20.0-29.0 (E)	No amplicon detected	Invalid
-	Cp > 33	Cp 20.0-32.5 (N) Cp 20.0-29.0 (E)	No amplicon detected	Invalid
-	-	Cp 20.0-32.5 (N) Cp 20.0-29.0 (E)	No amplicon detected	Invalid

*For reporting purposes these cases may be called for example "inconclusive" or "ambiguous".

To request our detailed instructions for use and for further information please contact our technical support at:
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