Eurofins Genomics Europe Synthesis GmbH

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

User Manual

REF 6100-ViroBO

100/1000 | 80°C | -20°C

For in-vitro diagnostic use only

For use with Roche LightCycler 480 II Instrument

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1. 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 (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*") specimen types by real time PCR systems. The ViroBOAR 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) 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, 5.1.2a health authorities preliminarily identified more than 40 human infections with a novel coronavirus in an outbreak of pneumonia under investigation in 5.1.2a 5.1.2a authorities identified a new type of coronavirus (novel coronavirus, named as 2019-nCoV), 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. The primer and probe design for this kit is based on the newly released strain (2019-nCoV) (GeneBank accession: MN908947) and covers six 2019-nCoV strains sequences (EPI_ISL_402119, EPI_ISL_402120, EPI_ISL_402121, EPI_ISL_402122, EPI_ISL_402123 and EPI_ISL_402124).
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).

4. Kit Contents

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

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.
Clinical specificity: 100 %
Clinical 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.

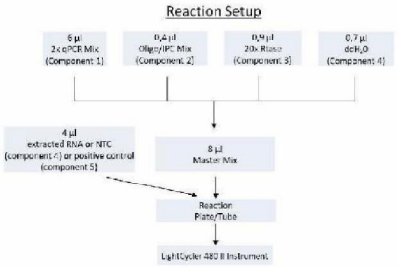
6. Additionally Required Materials and Devices
- Biological cabinet
- Vortex mixer
- Cryo-container
- Sterile filter tips for micro pipets
- Disposable gloves, powderless
- Refrigerator and freezer
- Real time PCR system
- Real time PCR reaction tubes/plates
- Pipets (0.5µl – 1000µl)
- Sterile microtubes
- Biohazard waste container
- Tube racks
- Desktop microcentrifuge for "Eppendorf" type tubes (RCF max. 16,000 x g)

7. Warnings and Precautions
- Carefully read this instruction before starting the procedure.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- Do not use the kit after 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/IPC Mix (component 2)
- 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 units.
- Use always sterile pipette tips with filters.
- 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 etiologic Agents

9. Procedure
9.1 RNA-Extraction
Different brand RNA extraction kits are available. You may use your own extraction systems or the commercial kits based on the yield. For the RNA extraction, please follow the manufacturer's instructions.
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)
The internal positive control in this kit contains a non-viral target sequence and is already contained in the Oligo/IPC mix. The IPC is intended to give signals in the HEX/VIC/Yellow555 channel.

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/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 (component 4) is set into the RT-PCR as no template control. Artificial RNA is used as positive control (component 5). For reasons of imprecise pipetting, always add an extra virtual sample. Mix completely and then spin down briefly with a centrifuge.
2) Pipet 8µl Master Mix with micropipetts of sterile filter tips to each of the Real Time PCR reaction plates/tubes. Separately add 4ul 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 in the instrument of **LightCycler 480 II:**

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	

10. Threshold Setting: Just above the maximum level of nuclease-free water.
11. Quality Control: Negative Control and Positive Control must be performed correctly; otherwise the sample results are invalid.

12. Data Analysis and Interpretation
The following sample results are possible:

2019nCoV_N1	2019nCoV_E	positive Control (1000 copies/RT-PCR), mean values of replicates	IPC (50,000 copies/RT-PCR)	Report
Cp < 38	Cp < 38	Cp 26.5-32.5 (N1) Cp 24.0-27.0 (E)	Cp > 20 or sigmoid curve	Positive 2019-nCoV
Cp < 38	-	Cp 26.5-32.5 (N1) Cp 24.0-27.0 (E)	Cp > 20 or sigmoid curve	ambiguous
-	-	Cp 26.5-32.5 (N1) Cp 24.0-27.0 (E)	Cp > 20 or sigmoid curve	Negative 2019-nCoV
Cp < 38	Cp < 38	Cp 26.5-32.5 (N1) Cp 24.0-27.0 (E)	Cp > 30	Positive 2019-nCoV
Cp < 38	-	Cp 26.5-32.5 (N1) Cp 24.0-27.0 (E)	Cp > 30	ambiguous
-	-	Cp 26.5-32.5 (N1) Cp 24.0-27.0 (E)	Cp > 30	Negative 2019-nCoV
Cp < 38	Cp < 38	Cp 26.5-32.5 (N1) Cp 24.0-27.0 (E)	-	Positive 2019-nCoV
Cp < 38	-	Cp 26.5-32.5 (N1) Cp 24.0-27.0 (E)	-	ambiguous
-	-	Cp 26.5-32.5 (N1) Cp 24.0-27.0 (E)	-	Negative 2019-nCoV

To request our detailed instructions for use and for further informations please contact our technical support at: 5.1.2e @eurofins.com