Eurofins Genomics Europe Synthesis GmbH

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ViroBOAR Spike 1.0 RT-PCR Kit (SARS-CoV-2)

User Manual

REF 6000-ViroBoSPIKE

Σ 100/1000/5000

-20°C -80°C

For *in-vitro* diagnostic use only

For use with Roche LightCycler 480 II Instrument

Eurofins Genomics Europe Synthesis GmbH
www.eurofinsgenomics.com
Anzinger Str. 7a
85560 Ebersberg
Germany

1. Intended Use

The ViroBOAR SPIKE 1.0 RT-PCR Kit is used for simultaneous qualitative detection of SARS-CoV-2 S gene variants in codon 501 and 570 in 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"*) and already pretested positive by RT-PCR method. The ViroBOAR SPIKE 1.0 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. End of 2020 two SARS-CoV-2 strains were identified that show mutations in different positions of the S gene, coding for the spike protein. Some of these mutations lead to a higher infectivity. Especially mutation N501Y is identified as one root cause. This mutation is found in virus isolates in UK (known as virus variant B.1.1.7), in South Africa (virus variant B.1.351) and in Brazil (virus variant P1). The strains from South Africa and Brazil can be distinguished from the strain from the strain from UK by the mutation A570D
which is only present in the strain from UK.
The primer and probe design for this kit is based on the reference sequence from the SARS-CoV-2 isolate Wuhan-Hu-1 (NCBI database: NC_045512.2) published by Wu et al. (Wu,F., Zhao,S., Yu,B., Chen,Y.M., Wang,W., Song,Z.G., Hu,Y.,Tao,Z.W., Tian,J.H., Pei,Y.Y., Yuan,M.L., Zhang,Y.L., Dai,F.H.,Liu,Y., Wang,Q.M., Zheng,J.J., Xu,L., Holmes,E.C. and Zhang,Y.Z. A new coronavirus associated with human respiratory disease in China. Nature 579 [7798], 265-269 [2020]).

The kit contains a specific ready-to-use system for the detection of Coronavirus SARS-CoV-2 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 (for the detection of S gene mutation N501Y and A570D) and HEX (for the detection of S gene "wildtype" version of N501Y and A570D) with MGBEQ Quencher.
Depending on user preference, the assays for codon 501 and 570 can be detected together in a duplex reaction or separately in single reactions.
This kit is intended to use for confirmatory analysis of extracts that have already tested positive for the presence of SARS-CoV-2 RNA only.

4. Kit Contents

Component Nr.	Kit Components	Presentation (100 rxns)	Presentation (1000 rxns)	Presentation (5000 rxns)
1	2x qPCR Mix	1 vial; 700 µl	1 vial; 7.0 ml	1 vial; 35 ml
2	Oligo Mix 501	1 vial; 20 µl	1 vial; 200 µl	1 vial; 1.0 ml
3	Oligo Mix 570	1 vial; 20 µl	1 vial; 200 µl	1 vial; 1.0 ml
4	20x Rtase	1 vial; 100 µl	1 vial; 1000 µl	1 vial; 5.0 ml
5	ddH20	1 vial; 110 µl	1 vial; 1100 µl	1 vial; 5.5 ml

There is no positive control for virus variants included in the kit. Therefore, it is the user's responsibility to select and use a suitable control for the test.

Based on data from validation study performed with 291 clinical samples (representing the different strains) pretested with another qPCR assay or with Next Generation Sequencing (NGS) analyses the following properties of the kit could be determined:

Limit of detection: The limit of detection of S gene mutants or wildtype is comparable to limit of detection of E gene if sample is analysed with ViroBOAR 4.0 kit.
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: 100 %
Clinical accuracy: 100 %

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 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 Mixes (Component 2 and Component 3)
• 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

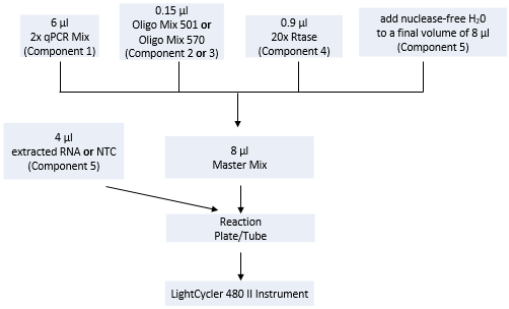
9.1 RNA-Extraction

During kit development and validation the PurePrep Pathogen Kit from Molgen on the King Fisher Flex instrument (Thermofisher Scientific) was used.
Please note 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.

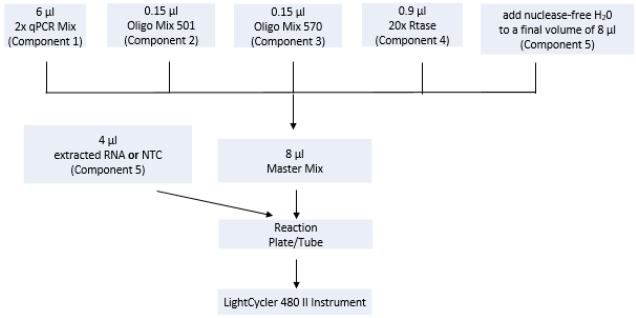
9.2 RT-PCR Protocol

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

Singleplex reaction:



Duplex-reaction:



1) Multiply the volumes of 2xqPCR Mix (Component 1), Oligo Mix (Component 2 and/or Component 3), 20xRtase (Component 4) and ddH2O (Component 5) per reaction with the number of planned PCR reactions, which includes the number of controls (NTC, positive control), and RNA extracts from patient samples prepared. ddH2O (Component 5) is set into the RT-PCR as no template control. 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. As positive and negative controls are not included in this kit, please follow the instructions of your respective supplier of controls.

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

The discrimination of S gene mutation on codon 501 is detected in the following channels:

N501Y

Target:	S Mutant	S Wildtype
Dye:	FAM	HEX
Wildtype	no signal	signal
B 1.1.7	signal	no signal
B.1.351 and P1	signal	no signal

The discrimination of mutation on codon 570 is detected in the following channels:

A570D

Target:	S Mutant	S Wildtype
Dye:	FAM	HEX
Wildtype	no signal	signal
B 1.1.7	signal	no signal
B.1.351 and P1	no signal	signal

If both assays for discrimination of mutation on codon 501 and 570 are used in combination signals must be detected in the following channels

N501Y / A570D

Target:	S Mutant	S Wildtype
Dye:	FAM	HEX
Wildtype	no signal	signal
B 1.1.7	signal	no signal
B.1.351 and P1	signal	signal

These duplex assays have been validated at Eurofins Genomics on the Roche LC 480 II**. For other instruments there are currently no analysis data available at Eurofins Genomics**. Discrimination of virus strains with respect to S gene codons 501 and 570 is only valid for samples which were already pretested positive for the presence of SARS-CoV-2 RNA and if the Cp value in one or both of the S gene channels is below 34.5.

Whole Genome Sequencing data generated during validation showed that at timepoint of validation also other strains as B.1.351, B.1.1.7 or P1 show mutations in one or both of the S gene variants analysed with this kit. Therefore, it must clearly stated that positive signal in one or both of the S gene channels not automatically can be correlated to a specific strain.

To request our detailed instructions for use and for further information please contact our technical support at:
E-Mail covid-eu@eurofins.com
Phone +49 7531 81 60 75 017
Fax +49 8092 8289 21084