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

User Manual

For research use only

For use with Roche LightCycler 480 II Instrument

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

100/1000/5000 REF -80°C -20°C **6000-ViroBoSPIKE**

Contents

1. Introduction

End of 2019, a novel respiratory disease emerged in the city of Wuhan, Hubei Province of the People's Republic of China, 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).

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

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) and in South Africa (virus variant B.1.351). These two strains can be distinguished by the mutation A570D which is present only in the strain from UK.

2. 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 for discrimination of SARS-CoV-2 wildtype virus and strains B1.1.7 and B1.351 (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.

3. Principle of the RT-PCR Assay

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.

Since this kit is used for confirmatory analysis of extracts that have already tested positive for the presence of corona virus RNA, it does not include a positive control that would detect inhibition.

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 increasing fluorescence signal generated during the PCR reaction 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 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

5. Stability and Storage

The ViroBOAR SPIKE 1.0 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 Mixes (Comp. 2 and Comp. 3) 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
- Viral RNA extraction kit (e.g. PurePrep Pathogen from Molgen)

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 SPIKE1.0 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. During kit development and validation, the *PurePrep Pathogens Kit* from Molgen was 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.

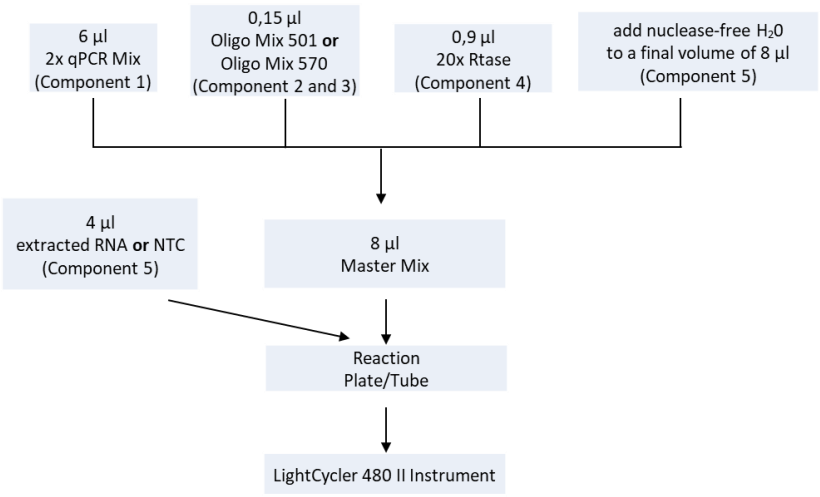
8. Assay Procedure

8.1 Reaction Setup

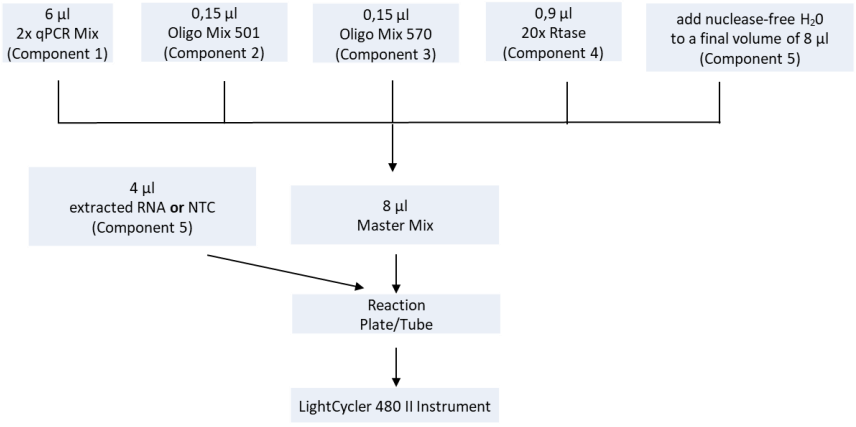
- Please read the instructions for use carefully before performing the assay. Reliability of results depends on following strictly the instructions for use.
- Before use make sure that all samples and reagents are thawed completely, mixed by up and down pipetting or vortexing and centrifuged briefly.
- Prepare quickly the Reaction Mix on ice or in the cooling block.
- If possible, run samples and controls in duplicates or triplicates. Pipette kit components slowly and carefully and use pipette tips suitable for pipetting viscous liquids.
- The use of ddH_2O (Comp. 5) 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 (Comp. 1), Oligo Mix 501 (Comp. 2), Oligo Mix 570 (Comp. 3), 20xRTase (Comp. 4), ddH_2O (Comp. 5) per reaction with the number of planned PCR reactions, which includes the number of NTCs and RNA extracts from patient samples prepared. ddH_2O (Comp. 5) is set into the RT-PCR as no template control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely and then spin down briefly with a centrifuge.

- Pipette 8µl Master Mix with a micropipette and sterile filter tips to each of the real-time PCR reaction plates/tubes. Separately add 4µl template (nucleic acid extracted from negative control (NTC) or RNA extracts from patient samples, positive control RNA with no extraction) to different reaction plates/tubes. Immediately close the plates/tubes to avoid cross-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.

<u>Singleplex-reaction:</u>



<u>Duplex-reaction:</u>



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:

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

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	

Fluorescent Detectors/Dyes:

Detection	Gen	Dye	Quencher	Detection channel (Excitation/Emission)
SARS-CoV-2	S gene: N501Y mutation	FAM	MGBEQ	465nm/510nm
SARS-CoV-2	S gene: N501Y wildtype	HEX	MGBEQ	533nm/580nm
SARS-CoV-2	S gene: A570D mutation	FAM	MGBEQ	465nm/510nm
SARS-CoV-2	S gene: A570D wildtype	HEX	MGBEQ	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.

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 probes for the "wildtype" sequences are HEX-labelled, the probes for the mutated sequences are FAM labelled.

If assay for discrimination of mutation on codon 501 is used in single confirmation assay without other controls signals must be 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	signal	no signal

If assay for discrimination of mutation on codon 570 is used in combination with the existing qPCR for detection of N gene and E gene signals must be 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	no signal	signal

If both assay for discrimination of mutation on codon 501 and 570 are used in combination with the existing qPCR for detection of N gene and E gene 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	signal	signal

These duplex assays can be used on all qPCR instruments that can measure into a FAM channel as well as a VIC/HEX channel. Besides Roche and Agilent, these are for example the instruments from e.g. Bio-RAD and Thermo Scientific. These duplex assays have also been used at Eurofins Genomics so far only 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.

10. Specific Performance Characteristics

Specific performance characteristics were determined with the Roche LightCycler 480 II in accordance with the manufacturer's recommendations.

Other commercially available SARS-CoV-2 kits were used as reference method for the evaluation of performance characteristics.

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

The use of other extraction kits may only be released after internal validation and after consultation of the kit manufacturer.

The use of other RealTime Instruments and resulting cut offs may only be released after internal validation and after consultation of the kit manufacturer.

Validation Parameter	Results	Acceptance criteria	Status
Specificity / Sensitivity	100 % fulfilled	100 % of all samples pretested or confirmed by NGS as wildtype or mutant must be determined as wildtype or mutant by qPCR	Passed
Limit of Detection	100% fulfilled	100 % of all samples which show a Cp value of 34,5 or lower with E gene assay must show a signal with S gene assay	Passed
Linearity	The correlation coefficient R^2 is ≥ 0,98.	The correlation coefficient R^2 must be ≥ 0,98	Passed

Accuracy	100 % fulfilled	The accuracy must be 100%: for the pretested or NGS confirmed "mutant" samples, no false "wildtype" results must be produced on these samples. For the NGS confirmed "wildtype" samples, no false "mutant" results must be produced on these samples.	Passed
Precision	100% fulfilled	The same RNA extracts must be determined in 100 % of the qPCR analyses as "wildtype" or "mutant".	Passed
Matrix Effect	100% fulfilled	All positive samples must be determined as "wildtype" or "mutant" in both assays. All samples must be positive or negative for Corona virus, respectively in both assays. Cp values should differ by not more than 3 Cp values.	Passed

The validation results are based on a sample size of 291 patient samples, among which 190 patient sample were tested positive for SARS-CoV-2 N gene and E gene.

11. Quality Control

In accordance with Eurofins Genomics Europe Synthesis GmbH Quality Management System (ISO 13485:2016), each lot of the ViroBoSPIKE 1.0 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)

PurePrep Pathogens Kit from Molgen (Utrecht, Netherlands; Art.No.OE00290096)

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 equipment than listed in “10. Specific Performance Characteristics” needs to be validated prior to routine diagnostic application.
- 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 beyond 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 sterile 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 re-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 kit components 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 (*PurePrep Pathogens Kit* from Molgen), 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 Mixes (Comp. 2 and Comp. 3)

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-ViroBoSPIKE	Corona Conformation Assay 1.0 RT-PCR Kit, 100 rxns
	6200-ViroBoSPIKE	Corona Conformation Assay 1.0 RT-PCR Kit, 1000 rxns
	6300-ViroBoSPIKE	Corona Conformation Assay 1.0 RT-PCR Kit, 5000 rxns

16. Bibliography

Bennett S, Davidson RS, Gunson RN. Comparison of gargle samples and throat swab samples for the detection of respiratory pathogens. J Virol Methods 2017; 248:83–6.

Gargle Lavage as a Safe and Sensitive Alternative to Swab Samples to Diagnose COVID-19: A Case Report in Japan Clin Infect Dis . 2020 Jul 28;71(15):893-894. doi: 10.1093/cid/ciaa377.

Gorbalenya, Alexander E.; Baker, Susan C.; Baric, Ralph S.; Groot, Raoul J. de; Drosten, Christian; Gulyaeva, Anastasia A. et al. (2020): Severe acute respiratory syndrome-related coronavirus: The species and its viruses – a statement of the Coronavirus Study Group (14). bioRxiv 2020.02.07.937862; doi: https://doi.org/10.1101/2020.02.07.937862

Gralinski, Lisa E.; Menachery, Vineet D. (2020): Return of the Coronavirus: 2019-nCoV. In Viruses 12 (2). DOI: 10.3390/v12020135.

RKI (Ed.): SARS-CoV-2 Steckbrief zur Coronavirus-Krankheit-2019 (COVID-19). Available online at https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Steckbrief.html. (accessed: 20.01.2021)

RKI (Ed.): Hinweise zur Testung von Patienten auf Infektion mit dem neuartigen Coronavirus SARS-CoV-2. Available online at https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Vorl_Testung_nCoV.html. (accessed: 20.01.2020)

Wang, Guan; Jin, Xian (2020): The progress of 2019 novel coronavirus event in China. In Journal of medical virology 92 (5), pp. 468–472. DOI: 10.1002/jmv.25705.

WHO: Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected. Interim guidance. 27 May 2020. Available online at https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected, accessed: 20.01.2020.

WHO: Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance, 19 March 2020. Available online at https://www.who.int/publications/i/item/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117, accessed: 20.01.2021.

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).

Zhou, Peng; Yang, Xing-Lou; Wang, Xian-Guang; Hu, Ben; Zhang, Lei; Zhang, Wei et al. (2020): A pneumonia outbreak associated with a new coronavirus of probable bat origin. In Nature 579 (7798), pp. 270–273. DOI: 10.1038/s41586-020-2012-7.

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

Eurofins Genomics Europe Synthesis GmbH
Anzinger Str. 7a
85560 Ebersberg
Germany
Internet: eurofinsgenomics.com
Email: support-eu@eurofins.com

Revision No.: EGE210122 / Issue Date: Jan 22th, 2021