EN

Instructions for Use

ViroQ SARS-CoV-2

Test kit for the detection of SARS-CoV-2 RNA

Electronic instructions for use see www.bag-diagnostics.com

RUO

REF 728261 ViroQ SARS-CoV-2 RUO 96 Test

REF 728262 ViroQ SARS-CoV-2 RUO 480 Test

For use with		
Specimen Types	**RNA extraction kits / automated extraction instruments**	**Real-time PCR instruments**
Nasopharyngeal (NP) swabs	QIAGEN QIAamp Viral RNA Mini Kit	Bio-Rad CFX96 Touch™ Real-Time PCR Detection System
Oropharyngeal (OP) swabs	QIAGEN QIAamp Viral RNA Mini QIAcube Kit / QIAcube	Roche LightCycler® 480 System II
Nasal swabs	QIAGEN QIAsymphony DSP Virus/ Pathogen Mini Kit / QIAsymphony SP	Applied Biosystems QuantStudio™ 6 Flex Real-Time PCR-System 96-Well Fast, laptop
Anterior nasal swabs	Roche Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit / MagNA Pure 96 Instrument	
Mid-turbinate nasal swabs		

Important Note: **For some cyclers a color compensation or dye calibration is needed to run the ViroQ SARS-CoV-2 test. Please check the continually updated list on our website via the button "Cycler settings": https://www.bag-diagnostics.com/en/sars-cov-2-en.html**

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Contents

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1. APPLICATION

The ViroQ SARS-CoV-2 Kit is used for qualitative detection of SARS-CoV-2 RNA in respiratory specimens such as nasopharyngeal (NP), oropharyngeal (OP), nasal, anterior nasal and mid-turbinate nasal swab based on reverse transcription of the RNA and subsequent amplification in real-time PCR.

For research use only – not for diagnostic purposes.

2. PRODUCT DESCRIPTION

The ViroQ SARS-CoV-2 Kit is used for the detection of SARS-CoV-2 RNA in respiratory specimens such as nasopharyngeal (NP), oropharyngeal (OP), nasal, anterior nasal and mid-turbinate nasal swab. The kit is based on a one step reaction with real-time PCR technology. An efficient cDNA synthesis from RNA coupled with a real-time PCR the ViroQ SARS-CoV-2 Kit makes it possible to perform the test in one tube. The kit is containing primers and fluorescent probes to amplify and detect gene fragments for SARS-CoV-2. In addition, it contains an internal control securing that the sampling of respiratory specimen was performed correctly and that the amplification worked.

3. TEST PRINCPLE

The test is performed with RNA as starting material. The RNA is converted into cDNA with a reverse transcriptase enzyme and afterwards amplified in a PCR. The primers were designed for the selective amplification of the trancripted cDNA of the viral genes RdRP and E (RdRP Gen: Institut Pasteur Protocol: Real-time RT-PCR assays for the detection of SARS-CoV-2. 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; E Gen: Corman et al. 2020). The amplicons are detected with likewise SARS-CoV-2 specific fluorescent dye-labelled hydrolysis probes (TaqMan® probes).

If amplicons are present, the probes are hydrolyzed by the Taq polymerase and a fluorescence signal is generated that increases proportionally to the amount of the PCR product. The fluorescence signals are measured by the optical detection unit of the RT-PCR-Cycler.

The test is performed in a single PCR reaction that detects the two viral genes RdRP and E and an universally expressed human housekeeping gene (Rnase P) with different flourescent colors. The detection of Rnase P indicates the correct sampling, RNA-Isolation and RT-PCR-amplification.

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4. MATERIAL

4.1 Content of the ViroQ SARS-CoV-2 kit

- **ViroQ|ENZYME** **ViroQ Enzyme**, lyophilized, contains Reverse Transcriptase, Taq Polymerase, dNTPs
- **ViroQ|SOLV** **ViroQ Solvent,** ready to use, contains reconstitution buffer for the ViroQ Enzyme
- **ViroQ|MIX** **ViroQ Mix**, ready to use, contains, Primers, Probes, Storage buffer
- **CONTROL|+** **ViroQ Pos Ctrl**, positive control, dryed, contains human mRNA, Virus reference RNA
- **IFU** **or** **eIFU** **Instructions for use or electronic instructions for use**

4.2 Additionally required reagents and devices

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- Reagents for RNA isolation (validated RNA isolation kits see 6.2)
- Real-time PCR-Cycler (validated cyclers see 4.3)
- RT-PCR reaction tubes with caps or foils (validated products see 4.3)
- RNase free H_2O
- Piston pipettes (0,5 – 1000 µl) and tips
- Color Compensation kit for LightCycler® 480 I+II, 2.0 (REF 728258 ViroQ CC Light Cycler®, provided by BAG Diagnostics)
- Color Calibration Kit for QuantStudio, StepOne, ABI 7500, ViiA7 (REF 728260 RT CC Universal Appplied Biosystems®, provided by BAG Diagnostics)

4.3 Validated cyclers and reaction tubes

Cycler	**real-time-PCR reaction tubes**	**real-time-PCR closing system**
CFX96 Touch™ Real-Time PCR Detection System Comp. Bio-Rad	FrameStar® Break-A-Way PCR Plate, Low Profile, 96 white wells, black frame Product No. 4ti-1201 Comp. 4titude / Brooks Life Sciences	Crystal Strips™ Product No. 4ti-0755/120 Comp. 4titude / Brooks Life Sciences
		qPCR Seal Product No. 4ti-0560 Comp. 4titude / Brooks Life Sciences
	Hard-Shell® 96-Well PCR Plates, Low Profile, thin wall, skirted, white/white Product No. HSP9655 Comp. Bio-Rad	0.2 ml Flat PCR Tube 8-Cap Strips, optical, ultraclear, Product No. TCS0803 Comp. Bio-Rad
LightCycler® 480 System II Comp. Roche	LightCycler® 480 Multiwell Plate 96, white Product No. 04729692001 Comp. Roche	qPCR Seal Product No. 4ti-0560 Comp. 4titude / Brooks Life Sciences
QuantStudio™ 6 Flex Real-Time PCR-System 96-Well Fast, laptop Comp. Applied Biosystems	Vari-Strip™ 8 Well PCR Tube Strips Product No. 4ti-0753 Comp. 4titude / Brooks Life Sciences	Crystal Strips™ Product No. 4ti-0755/120 Comp. 4titude / Brooks Life Sciences
	FrameStar® 96 Well Semi-Skirted, PCR Plate, ABI® FastPlate Style, white wells, clear frame Product No. 4ti-0911 Comp. 4titude / Brooks Life Sciences	qPCR seal, Product No. 4ti-0560 Comp. 4titude / Brooks Life Sciences

Special Note: If other realtime cyclers, reactions tubes and closing systems are used they must be validated by the user.

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5. STORAGE AND STABILITY

The kits are shipped at ambient temperature. Upon receipt store all reagents in temperature monitored devices at ≤ -20 °C. The expiry date is indicated on the label of each reagent. The expiry date indicated on the outer label refers to the reagent with the shortest stability contained in the kit. The reagents ViroQ Enzyme and ViroQ Solvent can be stored at room temperature until expiry date, as long as the enzyme lyophilisate is not solved with the reconstitution buffer. After solving it can be used upon 12 month. Repeated thawing and freezing of reagents (more than twice) should be avoided, as this might affect the performance of the assay. In terms of intermittent use the reagents should be aliquoted.

6. TEST PROCEDURE

6.1 Safety conditions and special remarks

Molecular genetic techniques are particularly sensitive and should be performed by well trained personnel experienced in molecular genetic techniques.

Special safety conditions must be observed in order to avoid contamination and thus false reactions:

- Wear gloves during work (powder-free, if possible).
- Use new tips with each pipetting step (with integrated filter).
- If possible, use separate working areas for pre-amplification (RNA isolation and PCR set up) and post-amplification (detection).
- Use devices and other materials only at the respective places and do not exchange them.

6.2 RNA Isolation

The sample material for the isolation of RNA must be sent in appropriate cell collection systems. For correct sampling follow the instructions given by the WHO under the following link https://www.who.int/csr/sars/sampling/en/

Validated RNA isolation kit:

Manual

- QIAGEN QIAamp Viral RNA Mini Kit

Automated

- QIAGEN QIAamp® Viral RNA Mini QIAcube Kit
- QIAGEN QIAsymphony® DSP Virus/Pathogen Mini Kit
- Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit

If the established standard method of the lab is used for RNA isolation and this is not the above mentioned kit, it must be validated by the user.

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6.3 Reagent preparation

ViroQ Enzyme

The enzyme mix reagent ViroQ Enzyme is lyophilized. Before use dissolve ViroQ Enzyme with 400 µl ViroQ Solvent by pipetting up and down.

ViroQ Pos Ctrl

The positive control reagent ViroQ Pos Ctrl is dried. Before use dissolve ViroQ Pos Ctrl with 30 µl RNasefree H_2O by pipetting up and down, allow complete rehydration for 15 minutes and then mix thoroughly by vortexing.

6.4 Amplification

Reaction tubes recommended by the manufacturer of the realtime cycler or the materials recommended in chapter 4.3 should be used.

For each sample the following reagents are pipetted into a reaction tube:

4 µl	ViroQ Enzyme
2 µl	ViroQ Mix
5 µl*	RNA Sample
9 µl	RNase free H_2O

*In case of very low expected concentration of virus copies the volume of the sample can be increased, while decreasing the amount of water.

The reaction volume for each real-time PCR test is 20 µl.

If a premix of ViroQ Enzyme and ViroQ Mix is prepared for more than one sample please allow for a reasonable additional amount for pipetting losses.

To perform the **positive control (PTC)** and a **no template control (NTC)** prepare a PCR reaction and use the ViroQ Pos Ctrl or water for the negative control instead of RNA.

Close the reaction tubes and briefly spin down the liquid. Ensure that no bubbles are present in the wells. If bubbles are observed, gently tap the reaction tube on the bench to remove the bubbles.

Start the PCR program with the following parameters:

Step	Time	Temperature	No. of cycles
Reverse Transcription	20 min	48°C	1 cycle
Polymerase activation	3 min	95°C	1 cycle
Denaturation	15 sec	95°C	45 cycles
Annealing + Extension	30 sec + reading	58°C	

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The following realtime cyclers have been validated for the ViroQ SARS-CoV-2 kit:

Biorad: CFX96 Touch™ Real-Time PCR Detection System

Roche: LightCycler® 480 System II

Applied biosystems: QuantStudio™ 6 Flex Real-Time PCR-System 96-Well Fast, laptop

Special Note

- If other realtime cyclers are used they have to be validated by the user.

6.5 Interpretation of results

For all three reactions in the multiplex PCR mix a Ct cutoff is used to define positive reactions. If the Ct-value is inconclusive it can be helpful to review the fluorescent curves.

All tests, except the negative control (NTC), must show a fluorescence signal in the red channel with the internal control. SARS-CoV-2 positive samples must show a positive signal in the FAM Channel (RdRP gene) or in both channels FAM and CFO560 / HEX / VIC / JOE channel (E gene). The positive control must show an amplification signal in each channel within the defined Ct-values.

Channel	**Specificity**
FAM	SARS-CoV-2 / RdRP Gene (**R**NA-**d**ependend **R**NA-**P**olymerase)
CFO560 / HEX / VIC / JOE	Beta-CoV / E Gene (Sarbeco, **E**nvelope)
CFR610 / Texas Red / ROX	Cell control / Rnase P

The amplification signals for SARS-CoV-2 negative samples should be outside the defined Ct-values for both channels (green and orange).
The negative control (NTC) is used as contamination control. If RNA or contaminating amplicon is inadvertently added to the NTC reaction a positive signal will occur. If the Ct is less than 35 it should be considered as possible contamination. Amplification signals above Ct 35 in the NTC could be PCR artefacts and can be disregarded taking into consideration the final RFU and the shape of the curve (see also below for interpretation of results between Ct 35 and Ct 45). If PCR contamination is suspected, it is advisable to follow local decontamination guidelines and to exchange the reagents.

For valid results all Ct values ≤ 35 are rated as positive (see table below).

	Channel	**Ct-Level**	**Inspect**	**Wave lenght in nm**
Cell control	Red (CFR610)	≤35*	>35-45**	Excitation: 590 Emission: 610
Virus Gene RdRP	Green (FAM)	≤35	>35-45	Excitation: 495 Emission: 520
Virus Gene E	Orange (CFO560)	≤35	>35-45	Excitation: 538 Emission: 559

* A high SARS-CoV-2 RNA concentration/load in the sample can lead to reduced or absent Cell control signals.

** Insufficient concentration/load of human cell material. Inappropiate sampling or sample shipment.

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Regardless of the Ct values a positive reaction should have a sigmoidal curve and a sufficient end RFU. The RFU is cycler dependent – the final RFU of the positive control can be used to get the approximate value that is normal for the final RFU on a given cycler. The positive control can also be used as an example for the correct sigmoidal shape of the curve. Therefore, samples with a Ct value of > 35 and low RFU should be checked for a sigmoidal shape of the curve and the plausibility of the reaction. Samples with a inconclusive result should be repeated. If there are questions regarding the adaptation of the threshold or borderline Ct values please contact the technical support of BAG Diagnostics (phone: +49 (0)6404 925125, email: info@bag-diagnostics.com) or your local sales representative.

The following table shows the interpretation of the amplification results:

FAM RdRP gene	CFO560 E gene	CFR610 cell control	Result
+	+	+*	SARS-CoV-2 specific RNA detected.
+	-	+*	SARS-CoV-2 specific RNA detected.
-	+	+*	Beta-CoV specific RNA detected. Repeat the test with the same or a new sample.
-	-	+	SARS-CoV-2 specific RNA not detected. The sample does not contain detectable or sufficient amounts of copies (LoD) of specific RNA.
-	-	-**	Invalid result due to real-time PCR inhibition or reagent failure. Repeat RNA isolation and/or testing from original sample.

* A high SARS-CoV-2 RNA concentration/load in the sample can lead to reduced or absent cell control signals.

** Insufficient concentration/load of human cell material. Inappropiate sampling or sample shipment.

7. WARNINGS AND PRECAUTIONS

ViroQ SARS-CoV-2 is designed for research use only and should be used by properly trained, qualified staff only. All work should be performed using Good Laboratory Practices.

The reagent ViroQ Solvent is subject to hazardous substance labeling for **Warning** and **Health hazard**. Please refer to the table in Chapter 12 for more information.

Biological material used for extraction of RNA, e.g. respiratory specimen, should be handled as potentially infectious. When handling biological material appropriate safety precautions are recommended (do not pipet by mouth; wear disposable gloves while handling biological material and performing the test; disinfect hands when finished the test).

Biological material should be inactivated before disposal (e.g. in an autoclave). Disposables should be autoclaved or incinerated after use.

Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated areas swabbed with a suitable standard disinfectant or 70% alcohol.

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Material used to clean spills, including gloves, should be inactivated before disposal (e.g. in an autoclave).

Disposal of all samples, unused reagents and waste should be in accordance with country, federal, state and local regulations.

Microbial contamination of the reagents while taking aliquots should be avoided. It is recommended to use sterile one way pipettes and tips. Reagents that look cloudy or show any signs of microbial contamination must not be used.

A Material Safety Data Sheet resp. a declaration on Material Safety Data Sheets (MSDS) is available to download at **www.bag-diagnostics.com**.

8. LIMITATIONS OF THE METHOD

Mutations or polymorphisms in the primer and probe binding sites may cause false negative results.
Because of the high susceptibility of the RT-PCR method for cross contaminations special care should be taken during RNA isolation.
The presence of PCR inhibitors may cause invalid results with this product. A negative result does not exclude a possible detection of SARS-CoV-2 RNA, as results are dependent on appropriate specimen collection, the absence of inhibitors and the defined LoD.
Extreme care should be taken to prevent contamination of the kit reagents and other laboratory materials and equipment with amplicons, RNA or DNA. Regular wipe tests and negative controls with Aqua dest with each assay are strongly recommended.
In the negative control with Aqua dest. there must not be any fluorescent signal (Ct > N.A.).
In the case of signal development in the negative control please refer to Chapter 6.5 and and if necessary, decontaminate the PCR working place and exchange the reagents.
All instruments (e.g. pipettes, realtime cyclers) must be calibrated according to the manufacturers instructions.
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9. INTERNAL QUALITY CONTROL

Internal quality control of new lots of the ViroQ SARS-CoV-2 kit can be performed using a combination of RNA samples known to be positive or negative. Negative controls to detect possible contaminations are recommended. Use a PCR reaction with the RNase free water as a NTC for this purpose.

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10. TROUBLESHOOTING

Symptom	Possible reason	Potential solution
Bad or no signal	Presence of an inhibitor.	Use fresh reagents.
	No RNA in the reaction.	Repeat test. Take care of correct pipetting.
	Fluorescent probes or primers degraded.	Use fresh Primer-Probe-Mix Avoid exposition to light and frequent thawing and freezing. Observe storage conditions!
	Bubbles in the PCR reaction, remaining liquid at the inner wall of the tube.	Careful pipetting. Spin down PCR plate.
	Incompatible or low quality RT-PCR plastic ware.	Use compatible and high quality plastic ware (see chapter 4.3).
	Evaporation of the reagents due to incorrect closing of the PCR tubes.	Make sure that the PCR tubes are closed properly. Be careful at the edges of sealing foils.
Signal in the negative control	Contamination with RNA or DNA in the negative control	Repeat the negative control. Decontaminate the workplace.

11. TRADEMARKS USED IN THIS DOCUMENT/PRODUCT

TaqMan® is a trademark of Roche Molecular Systems Inc.
Cal Fluor® is a registered trade mark of LGC Biosearch Technologies

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12. EXPLANATION OF SYMBOLS USED ON THE LABELS

	Sufficient for n tests
	Storage temperature / Lower limit of temperature
	Use by
	Consult instructions for use
	Manufacturer
DRY	Dried
CONT	Content, contains
CONTROL | +	Positive control
IFU	Instructions for use
or	or
eIFU	Electronic instructions for use
LOT	Batch code
LYOPH	Lyophilized
REF	Catalogue number
RUO	For Research Use Only
ViroQ | ENZYME	Enzyme mix for ViroQ products
ViroQ | MIX	Primermix for ViroQ products
ViroQ | SOLV	Solvent for ViroQ enzyme mix
	**Warning** H302: Harmful if swallowed. H412: Harmful to aquatic life with long lasting effects.
	**Health hazard** H371: May harm the central nervous system. Route of exposure: Oral

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13. LITERATURE

Victor M Corman, Christian Drosten et.al.(2020), Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, Euro Surveill. 2020;25(3):pii=2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045

Institut Pasteur Protocol: Real-time RT-PCR assays for the detection of SARS-CoV-2. 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

Further information is provided on our website http://www.bag-diagnostics.com.

Instructions for use in other languages see: http://www.bag-diagnostics.com or contact us directly at info@bag-diagnostics.com or phone +49 (0)6404-925-125

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