EN

Instructions for Use

ViroQ SARS-FluA/B-RSV

Test kit for the qualitative detection of SARS-CoV-2, Influenza A, Influenza B and RSV RNA

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

RUO

REF 728269 ViroQ SARS-FluA/B-RSV 96 Tests

REF 728270 ViroQ SARS-FluA/B-RSV 480 Tests

For use with		
Specimen Types	**RNA extraction kits / automated extraction instruments**	**Real-time PCR instruments**
Nasopharyngeal (NP) swabs	QIAGEN EZ1 Virus Mini Kit V2.0 Thermo Fisher Thermo KingFisher MagMAX Viral/PathogenNucleic Acid Isolation Kit	Bio-Rad 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
Oropharyngeal (OP) swabs		
Nasal swabs		
Anterior nasal swabs		
Mid-turbinate nasal swabs		

Important Note: **For some cyclers a color compensation or dye calibration is needed to run the ViroQ SARS-FluA/B-RSV 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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1. Application

The ViroQ SARS-FluA/B-RSV Kit is used for qualitative detection of SARS-CoV-2, Influenza A, Influenza B and RSV (respiratory Syncytial Virus) 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-FluA/B-RSV Kit is used for the detection of SARS-CoV-2, Influenza A, Influenza B and RSV 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-FluA/B-RSV 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, Influenza A, Influenza B and RSV. In addition, it contains an internal control securing that the sampling of respiratory specimen was performed correctly and that the amplification worked.

3. TEST PRINCIPLE

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 transcripted cDNA of the viral genes. For SARS-CoV-2 the gene RdRP (RNA-dependend RNA-Polymerase) and N (Nucleocapsid) are amplified (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). For Influenza A the Matrix (M1), for Influenza B the nonstructural protein (NS1) and for RSV the N gene (Nucleocapsid) is amplified. The amplicons are detected with likewise 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 with the amount of the PCR product. The fluorescence signals are measured by the optical detection unit of the real-time PCR cycler.
The test is performed in a single PCR reaction that detects the two viral genes RdRP and N of SARS-CoV-2, the Matrix of Influenza A, the nonstructural protein of Influenza B, the N gene of RSV 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.

4. MATERIAL

4.1 Content of the ViroQ SARS-FluA/B-RSV kit

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- **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 | FSR** **ViroQ Mix FSR**, ready to use, contains, Primers, Probes, Storage buffer
- **ViroQ | CFX | IC | MIX** **ViroQ CFX IC-Mix**, contains Probe of the internal control with a special fluorophor suitable for the CFX cycler
- **ViroQ | LC | IC | MIX** **ViroQ LC IC-Mix**, contains Probe of the internal control with a special fluorophor suitable for the LightCycler®
- **ViroQ | QS | IC | MIX** **ViroQ QS IC-Mix**, contains Probe of the internal control with a special fluorophor suitable QuantStudio
- **ViroQ | FSR | CONTROL | +** **ViroQ Pos Ctrl FSR**, positive control, dryed, contains human RNA, Virus reference RNA
- **IFU** **OR** **eIFU** **Instructions for use or electronic instructions for use**

4.2 Additionally required reagents and devices

- Reagents for RNA isolation (validated RNA isolation kits see 6.2)
- Real-time PCR Cycler (validated cyclers see 4.3)
- Real-time 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 and Cobas z 480 Analyer (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

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Cycler	real-time-PCR reaction tubes	real-time-PCR closing system
CFX96 Touch™ Real-Time PCR Detection System Comp. Bio-Rad	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® Break-A-Way PCR Plate, Low Profile, 96 white wells, black frame Product No. 4ti-1201 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 real time cyclers, reactions tubes and closing systems are used they must be validated by the user.

5. STORAGE AND STABILITY

The kits are shipped without cooling. 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 already solved reagents (more than twice) should be avoided, as this might affect the performance of the assay. For intermittent use the reagents should be aliquoted.

6. TEST PROCEDURE

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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 sample 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 kits:

- QIAGEN QIAamp® Viral RNA Mini QIAcube Kit
- Thermo Fisher Thermo KingFisher MagMAX Viral/PathogenNucleic Acid Isolation 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.

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 FSR
The positive control reagent ViroQ Pos Ctrl FSR is dried. Before use dissolve ViroQ Pos Ctrl FSR with 30 µl RNase-free H2O 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.

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

4 µl	ViroQ Enzyme
2 µl	ViroQ Mix FSR (Primer and Probes)
2 µl	ViroQ CFX/LC/QS IC-Mix (Probe)
5 µl*	RNA Sample
7 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.

Special Note: The internal amplification control will be detected in different fluorescence channels depending on the real-time PCR cycler used for the run. Therefore the suitable ViroQ IC-Mix should be used, because it is containing the probe for the internal amplification control labelled with different fluorophors. Please check the filter of the real-time PCR cycler used. The wave length of the fluorophors are shown in a table in chapter 6.5 Interpretation of results.

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

If a premix of ViroQ Enzyme, ViroQ Mix FSR, ViroQ CFX/LC/QS IC-Mix and RNase free H_2O 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 FSR or RNAse free water for the NTC 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	15 min	48°C	1 cycle
Polymerase activation	3 min	96°C	1 cycle
Denaturation	3 sec	95°C	42 cycles
Annealing + Extension	15 sec + reading	60°C	

The following real-time cyclers have been validated for the ViroQ SARS-FluA/B-RSV 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.

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6.5 Interpretation of results

For all 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 channel with the internal control. SARS-CoV-2 positive samples must show a positive signal in the FAM channel (RdRP gene & N gene). Influenza positive samples must show a positive signal in either the CFO560 / HEX / VIC / JOE channel or in the Q670 / CY5 channel. RSV positive samples must show a positive result in the CFR610 / Texas Red / ROX channel. The positive control must show an amplification signal in each channel within the defined Ct-values.

Channel	Specificity
FAM	SARS-CoV-2 / RdRP Gen (**R**NA-**d**ependend **R**NA-**P**olymerase) & N Gen (Nucleocapsid)
CFO560 / HEX / VIC / JOE	Influenza A (H1N1pdm09 & H3N2) / Matrix (M1)
Q670 / CY5	Influenza B (Yamagata & Victoria) / nonstructural protein 1 (NS1)
CFR610 / Texas Red / ROX	RSV / N Gen (Nucleocapsid)
Atto425 / Q705 / TAMRA	Cell control / Rnase P

The amplification signals for the different specificities of negative samples should be outside the defined Ct-values for the corresponding channels.
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.

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

	Channel	Ct-Level	Inspect	Wave lenght in nm
RSV	Red (CFR610)	≤35	>35-42	Excitation: 690 Emission: 705
SARS-CoV-2	Green (FAM)	≤35	>35-42	Excitation: 495 Emission: 520
Influenza A	Orange (CFO560)	≤35	>35-42	Excitation: 538 Emission: 559
Influenza B	Red (Q670)	≤35	>35-42	Excitation: 647 Emission: 670
Cell control with ViroQ CFX IC-Mix	Red (Q705)	≤35*	>35-42**	Excitation: 590 Emission: 610
Cell control with ViroQ LC IC-Mix	Blue (Atto425)	≤35*	>35-42**	Excitation: 437 Emission: 483
Cell control with ViroQ QS IC-Mix	Red (TAMRA)	≤35*	>35-42**	Excitation: 557 Emission: 591

* A high virus 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.

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 and interpreted. 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.

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The following table shows the interpretation of the amplification results:

FAM SARS-CoV2	CFO560 Influenza A	Q670 Influenza B	CFR610 RSV	Atto425 / Q705 /TAMRA Zellkontrolle	Ergebnis
+	-	-	-	+*	SARS-CoV-2 specific RNA detected.
-	+	-	-	+*	Influenza A specific RNA detected.
-	-	+	-	+*	Influenza B specific RNA detected.
-	-	-	+	+*	RSV specific RNA detected.
+	+	-	-	+*	SARS-CoV-2 and Influenza A specific RNA detected.
+	-	+	-	+*	SARS-CoV-2 and Influenza B specific RNA detected.
+	-	-	+	+*	SARS-CoV-2 and RSV specific RNA detected.
-	+	+	-	+*	Influenza A and Influenza B specific RNA detected.
-	+	-	+	+*	Influenza A and RSV specific RNA detected.
-	-	+	+	+*	Influenza B and RSV specific RNA detected.
-	-	-	-	+	SARS-CoV-2, Influenza A, Influenza B or RSV 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 virus 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-FluA/B-RSV is designed for resarch use only purposes 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 13 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 and mouth-nose-protection 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.

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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.
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 the presence of SARS-CoV-2, Influenza A, Influenza B and RSV specific 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 no template 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, real time cyclers) must be calibrated according to the manufacturers instructions.

9. INTERNAL QUALITY CONTROL

Internal quality control of new lots of the ViroQ SARS-FluA/B-RSV kit can be performed using a combination of RNA samples known to be positive or negative for the different spcifities. 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 ViroQ 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
Quasar® is a registered trade mark of LGC Biosearch Technologies

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12. EXPLANATION OF SYMBOLS USED ON THE LABELS

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
ViroQ | FSR | CONTROL | +	Positive control for ViroQ SARS-FluA/B-RSV
IFU	Instructions for use
OR	or
eIFU	Electronic instruction for use
RUO	For research use only
LOT	Batch code
LYOPH	Lyophilized
REF	Catalogue number
ViroQ | ENZYME	Enzyme mix for ViroQ products
ViroQ | MIX | FSR	Primermix for ViroQ SARS-FluA/B-RSV
ViroQ | CFX | IC | MIX	Additional probe mix for ViroQ SARS-FluA/B-RSV when using Real-time PCR-Cycler CFX96 Touch™ Real-Time PCR Detection System
ViroQ | LC | IC | MIX	Additional probe mix for ViroQ SARS-FluA/B-RSV when using Real-time PCR-Cycler LightCycler® 480 System II
ViroQ | QS | IC | MIX	Additional probe mix for ViroQ SARS-FluA/B-RSV when using Real-time PCR-Cycler QuantStudio™ 6 Flex Real-Time PCR-System 96-Well Fast, laptop
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

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