EN

Instructions for Use

ViroQ SARS-CoV-2

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

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

CE IVD

REF 728250 ViroQ SARS-CoV-2 96 Tests

REF 728251 ViroQ SARS-CoV-2 480 Tests

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1. INTENDED USE

The ViroQ SARS-CoV-2 Kit is used for qualitative detection of SARS-CoV-2 RNA in respiratory specimens such as sputum or swabs based on reverse transcription of the RNA and subsequent amplification in real-time PCR. The test is performed by qualified personnel in specialised labs.

2. PRODUCT DESCRIPTION

The ViroQ SARS-CoV-2 Kit is used for the *in vitro* detection of SARS-CoV-2 RNA in respiratory specimens such as sputum and swabs. 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 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 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 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 E and an universally expressed human housekeeping gene (Rnase P) with different flourecent colours. The detection of Rnase P indicates the correct sampling, RNA-Isolation and RT-PCR-amplification.

4. MATERIAL

4.1 Content of the ViroQ SARS-CoV-2 kit

- **ViroQ Enzyme**, lyophilized, contains Reverse Transcriptase, Taq Polymerase, dNTPs
- **ViroQ Solvent,** ready to use, contains reconstitution buffer for the ViroQ Enzyme
- **ViroQ Mix**, ready to use, contains, Primers, Probes, Storage buffer
- **ViroQ Pos Ctrl**, dryed, contains human mRNA, Virus reference RNA
- **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)
- RT-PCR reaction tubes with caps or foils (validated products see 4.3)

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- DEPC H_2O / Aqua dest. / WFI (Ampuwa®)
- Piston pipettes (0,5 – 1000 µl) and tips

4.3 Validated cyclers and reaction tubes

Cycler	real-time-PCR reaction tubes	real-time-PCR closing system
CFX96™ Real-Time PCR Detection System Comp. Bio-Rad	FrameStar® Break-A-Way PCR Plate, 96 clear wells, clear frame, Product No. 4ti-1200/C Comp. 4titude	4titude Crystal Strips Product No. 4ti-0755 Comp. 4titude
		4titude qPCR seal, Product No. 4ti-0560 Comp. 4titude
	Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/white, #HSP9655 Comp. Bio-Rad	0.2 ml Flat PCR Tube 8-Cap Strips, optical, ultraclear, #TCS0803 Comp. Bio-Rad
LightCycler® 480 System Comp. Roche	LightCycler® 480 Multiwell Plate 96, white Product No. 04729692001 Comp. Roche	LightCycler® 480 Sealing Foil, Product No. 04729757001 Comp. Roche
QuantStudio 6 Comp. Applied Biosystems	4titude FrameStar 96 Well Semi-Skirted, PCR Platte, ABI FastPlate Style Product No. 4ti-0911 Comp. 4titude	4titude qPCR seal, Product No. 4ti-0560 Comp. 4titude
		4titude Crystal Strips Product No. 4ti-0755 Comp. 4titude

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 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 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

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.

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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/

It is recommended to use CE IvD certified kits for the RNA isolation.

Validated RNA isolation kits:

Manual

- QIAamp Viral RNA Mini Kit

Automated

- QIAamp® Viral RNA Mini QIAcube Kit
- QIAsymphony® DSP Virus/Pathogen Mini Kit
- Roche MagNA Pure 96 System

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

The enzyme mix ViroQ Enzyme must be dissolved prior use with 400 µl ViroQ Solvent by pipetting up and down.
The positive control ViroQ Pos Ctrl must be dissolved prior use with 30 µl Rnase free H_2O by pipetting up and down.

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 (Primer and Probes)
5 µl*	RNA Sample
9 µl	DEPC H_2O / WFI

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

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

If a premix of ViroQ Enzyme, ViroQ Mix and DEPC H_2O / WFI 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 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	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	

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

Biorad: CFX96™ Real-Time PCR Detection System

Roche: LightCycler® 480 II System

Applied biosystems: QuantStudio 6

Special Note

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

6.5 Interpretation of results

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 inside the defined Cq-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 Cq-values for both channels (green and orange). The NTC with Aqua dest. should not show any fluorescent signal during the complete real-time PCR run and represents a contamination control. Fluorescence signals with the NTC indicate contamination. Fluorescence signals outside the defined Cq values can occur due to the very sensitive test method in case of inaccurate pipetting. If this occurs, the test should be repeated.

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The following signals are rated as positive:

	Channel	Cq-Level	Inconclusive	Wave lenght in nm
Cell control	Red (CFR610)	≤40*	>40-45**	Excitation: 597 Emission: 610
Virus Gene RdRP	Green (FAM)	≤40	>40-45	Excitation: 495 Emission: 520
Virus Gene E	Orange (CFO560)	≤40	>40-45	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.

Cq-level is the PCR cycle that shows a positive detection against the background.

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 test.
-	-	+	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. SPECIFIC PERFORMANCE CHARACTERISTICS

The combination of primers and probes ensures a reliable identification of SARS-CoV-2 specific RNA. The accuracy and reproducibility of the specificity of the test kit is verified for each lot with pre-typed reference samples.

7.1 Limit of detection

The lowest SARS-CoV-2 RNA concentration that is successfully detected with a probability of 95% or higher defines the Limit of Detection (LoD). The LoD was evaluated with five different dilutions of a reference virus RNA which were each tested 20 times. According to this experiment the analytical sensitivity of the ViroQ SARS-CoV-2 RT-PCR is **5 copies / 20 µl reaction** for both target genes (RdRP, E gene).

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7.2 Clinical Evaluation

For the ViroQ SARS-CoV-2 kit a performance evaluation study was performed with 376 pre-typed RNA samples. The results from the study were compared to the results that were obtained with test kits from other manufacturers.

		ViroQ SARS-CoV-2	
		Positive	Negative
Pre-defined result	Positive	40	0
	Negative	1*	335

Diagnostic specificity: 99,7%
Diagnostic sensitivity: 100%

*inconclusive result, only the E gene has been detected in this sample!

7.3 Cross-Reactivity

Cross-reactivity of the primers with other respiratory viruses and bacteria was tested by the Pasteur Institute (Paris) and by Corman et al. 2020 with known positve samples. None of the tested organisms showed a reactivity.

To demonstrate the analytical specificity and exclusivity of the ViroQ SARS CoV-2 kit, a control panel containing 22 respiratory pathogens (intact virus particles and bacterial cells) was used. RNA was extracted from each pool contained in the panel (see table below) and tested with the ViroQ SARS-CoV-2 Kit. No pool showed reactivity with the RdRP gene. For the E gene a very weak reaction was detected for all pools.

Respiratory control panel					
	Pool 1	Pool 2	Pool 3	Pool 4	Pool 5
Adenovirus Type 3	✓				
Coronavirus OC43	✓				
Human Metapneumovirus (Peru6-2003)**	✓				
Parainfluenza Type 2	✓				
B. pertussis (A639)	✓				
Coronavirus NL63		✓			
Bocavirus-Lambda (recombinant, Isolate 2)		✓			
Influenza A H1 (A/New Caledonia/20/99)		✓			
Parainfluenza Type 3		✓			
Coronavirus 229E			✓		
Rhinovirus (1A)			✓		
Influenza A H3 (A/Brisbane/10/07)			✓		
C. pneumoniae (CWL-029)			✓		
Influenza B (B/Florida/02/06)				✓	
Parainfluenza Type 4A				✓	
Respiratory Syncytial Virus B (CH93(18)-18)				✓	
M. pneumoniae (M129)				✓	
Coronavirus HKU-1 (recombinant)				✓	
Influenza A H1N1 (A/NY/02/09)					✓
Parainfluenza Type 1					✓
Respiratory Syncytial Virus A (2006 Isolate)					✓
L. pneumophila (Philadelphia)					✓

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8. WARNINGS AND PRECAUTIONS

ViroQ SARS-CoV-2 is designed for in-vitro-diagnostic 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.

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**.

9. 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 infection, 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 (Cq > N.A.). In the case of signal development in the negative control the PCR working place has to be decontaminated and the reagents have to be exchanged if necessary.

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All instruments (e.g. pipettes, realtime cyclers) must be calibrated according to the manufacturers instructions.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

10. 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.

11. 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.

12. 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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13. 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
IVD	For in vitro diagnostic use
LOT	Batch code
LYOPH	Lyophilized
REF	Catalogue number
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

14. 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

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