ViroGene SARS-CoV-2 Direct Confirm Kit

Real Time PCR Qualitative Kit (Rev. 1.1)

Article No 31492, 31494 CE IVD

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ViroGene SARS-CoV-2 Direct Confirm

Real-Time RT-PCR Detection

A. General

- ViroGene® SARS-CoV-2 Direct Confirm RTU kits are intended for the Direct Detection of the novel SARS-CoV-2 in samples with positive results from ViroGene® SARS-CoV-2 Screening RTU. The kits are for in vitro diagnostic purposes and suitable for the analysis of samples from humans, such as naso-/oro-pharyngeal swabs collected in Appropriate Transport Medium (UTM, VTM. PBS and Saline)
- The qualitative testing with ViroGene® SARS-CoV-2 Direct Confirm RTU kits is based on a duplex Real-Time RT-PCR: In one reaction setting, the RNA target sequences for SARS-CoV-2 (RdRP and S-gene) as well as for the Exogenous control are reverse transcribed (Reverse Transcription (RT)) and amplified in parallel with respective primer pairs in the Polymerase Chain Reaction (PCR). Amplified target gene fragments are detected via fluorescently labeled probes during the PCR reaction in real-time (Real-Time PCR). The probes specific for detection of amplified SARS-CoV-2 (RdRP and S-gene) and the Exogenous control Target genes are labeled with fluorescent dyes FAM and VIC, respectively, and their emitted fluorescence is separately optically measured by the Real-Time PCR thermal cycler. By means of both individual analyses in one reaction vessel per sample and the Negative Control and Positive Control per run the SARSCoV-2-specific status of a sample can be evaluated in the end. This way, results can be achieved within a few hours after sample receipt.
- We recommend to test samples with ViroGene® SARS-CoV-2 Direct Screening RTU kits before using ViroGene® SARS-CoV-2 Direct Confirm RTU kits.
- These kits were developed for use by trained laboratory personnel following standardized procedures. This Direction for Use must be followed strictly. For in vitro diagnostic use only.
- The setup used for the specific detection in this kit includes a published setup listed by the World Health Organization (WHO).

B. Reagents and Materials

- The following ViroGene® SARS-CoV-2 Direct Confirm RTU kits are available and comprise the following reagents:

Reagent	100 Reactions Article No 31492 REF	25 Reactions Article No 31494 REF	Store at
RNA Buffer Mix	1 x 1250 µl	1 x 315 µl	≤ -18 °C
Positive Control	1 x 100 µl	1 x 100 µl	
Negative Control	1 x 1 ml	1 x 1 ml	
Primer Probe Mix	1 x 150 µl	1 x 37.5 µl	
RT Enzyme Mix	1 x 100 µl	1 x 25 µl	
Internal Control	2 x 1250 µl	1 x 250 µl	
Direct Lysis Buffer	1 x 1250 ul	625 ul	(2 - 8) °C

- After receipt, the components are immediately stored at ≤ -18 °C. Avoid repeated freezing and thawing of all the reagents and keep them thawed as short as possible. If occasional processing of few samples only is expected you may prepare appropriate aliquots of reagents before storage at ≤ -18 °C. Prepare aliquots in such a way that freeze-thaw-cycles are reduced to a maximum of three. The Direct Lysis Buffer and Neutralization Buffer can alternatively be stored at +2°C to +8°C.

- The components are to be used within the indicated shelf life (see box label). The components of different batches may not be mixed.

- The Reaction-Mix needs to be stored protected from abundant light. Do not expose to direct (sun)light.

C. Equipment and Reagents not included

- This detection method can be used on all commercially available Real-Time PCR thermal cyclers that detect the emitted fluorescence of the fluorescent dyes FAM and VIC (emission 520 and 550 nm, respectively). Note that default normalization option against ROX (e.g. using ABI cyclers) must be deactivated.

- We recommend the exclusive use of certified Nuclease-free disposables as well as powder-free protective gloves. Please wear gloves during the entire experimental procedure. Gloves need to be changed frequently, especially after spillage or suspected contaminations.

D. Control Reactions

- The Positive Control allows for control of the specificity and efficiency of the reagents and the reaction itself, including the performance of the Real-Time RT-PCR and of the Real-Time PCR thermal cycler.
- The Negative Control allows for exclusion of contaminations. The sample testing is only valid if both, Positive and Negative Controls, are used and valid in every Real-Time RT-PCR run.
- The Internal Control is based on detection of Artificial RNA, The Internal Control target is co-amplified (channel VIC) with every single reaction and allows for evaluation of sufficient sampling, sample storage and shipment, sample preparation and the Real-Time RT-PCR run itself.
- It is recommended to run one or more of an RNA Isolation Control (RIC) per set of RNA preparation, depending on the total number of samples processed at once. The RIC is a "mock sample" composed of the plain sterile buffer used for raw sample processing. It is randomly placed between the samples, processed like a normal sample and allows to detect potential contaminations of the reagents used (additionally to the Negative Control reaction) as well as for the detection of potential carryover contaminations between individual samples, e.g. during the RNA preparation process.

E. Protocol

- The overall protocol of the analysis consists of the following main workflow:

1. Sample Preparation
2. RNA Preparation
3. Reaction Setup, Reverse Transcription and Amplification (Real-Time RT-PCR)
4. Data Analysis – Validity and Qualitative Result

- We recommend proceeding through the protocol without interruption to avoid potential degradation of the processed samples and reagents. If necessary, you may store the final RNA preparation at ≤ -18 °C until further processing. Avoid repeated freezing and thawing of the RNA preparations.

1. Sample Preparation

- Let swabs soak in a sufficient volume of sterile buffer (e.g. 1 ml of Normal Saline or 0.1 x TE) for an adequate period of time and finally wash out the swabs by thorough pulse-vortexing.
- The supernatant is used for RNA preparation.
- Sample stability is dependable on type of Transport Medium used.

2. Direct SARS CoV-2 RNA Samples Preparation

- All kinds of sample Transport Medium (VTM, UTM, PBS, Saline) are supported by this Direct PCR Protocol
- for Laboratory Personal Safety it is recommended to Heat samples at 65 C for 10 Minutes to Heat Inactivate the Virus before proceeding.
- Samples may be processed in Water Bath or Heat Block.
  - If this step is to be used with large sample numbers, thermal cycler maybe used instead of the water bath with 70 C for 10 minuses followed by 2 minutes at 25 C to cool down samples, then proceed to the following steps.
- After the Heat Inactivation Step, please Cool down the samples to Room Temperature.
- Mix 10 ul of the Direct Lysis Buffer with 40 ul sample, mix well and let stand at Room Temperature for 5 min., in another tube mix 20 ul Internal Control with 10 ul of sample lysate and mix well with Internal Control. Use 10 ul in Reaction setup.

3. Reaction Setup and Amplification (Real-Time RT-PCR)

Number of Reactions (rxns)	1 SAMPLE
RNA Buffer Mix	12.5 µl
Primer and Probe Mix	1.5 µl
RT Enzyme Mix	1.0 µl
Volume Reaction-Mix	**15.0 ul**

- Before each use, briefly vortex and spin down the Reaction-Mix and positive Control.
- To determine the total number of reactions needed, count the number of samples and add two more for the Negative Control and the Positive Control.
- The Reaction-Mix is ready-to-use, add 15.0 µl to each of the PCR tubes or plate wells ("cavities")
- Keep exposure of the Reaction-Mix to (sun)light as short as possible and return it back to appropriate storage temperature right after application. Avoid the formation of bubbles when pipetting samples and controls.

- Add 10 µl of the Negative Control to the corresponding cavity and seal it individually, if possible.
- Add 10 µl of each RNA preparation to the corresponding cavities and seal them individually, if possible. To minimize risk of potential cross-contaminations,
- 10 µl of the Positive Control are added to the corresponding cavity after all previous samples and control reactions are set up. Before each use, briefly vortex and spin down the rehydrated Positive Control (see also chapter B "Reagents and Materials").
- If not already done, finally seal the cavities. It is recommended to briefly spin them down before the start of the Real-Time RT-PCR run.
- Place the cavities in the Real-Time PCR thermal cycler and run the test with ViroGene® Thermal Profile as given below.

ViroGene® Thermal Profile				
Step No	**Description**	**Temperature**	**Duration**	
1	Reverse Transcription	50 °C	10 min	
2	Activation of Polymerase	95 °C	10 min	
3	Denaturation	95 °C	15 sec	
4	Annealing & Extension	60 °C	30 sec	} 45 cycles
5	Fluorescence Detection	channels FAM and VIC		

- ViroGene® Thermal Profile allows for combined run of this and most other ViroGene® RT-qPCR detection methods as well as ViroGene® PCR detection products.
- In the event of a combined Real-Time (RT-)PCR run, make sure all necessary channels are detected.

Please follow the specified instructions of your Real-Time PCR thermal cycler as recommended by the manufacturer.

4. Data Analysis – Validity and Qualitative Result

General

- The amplification data can be processed automatically using the specific software tool of your Real-Time PCR thermal cycler. Alternatively, the threshold can be set manually considering the following directions: The threshold should cross the FAM and VIC curves in the exponential phase of the PCR reaction (best visible as a linear slope when the Y-scaling is set to logarithmic). By setting the threshold, the crossing points with the VIC- and FAM-curves determine the respective cycle threshold (Ct), which is negatively correlated with the initial concentration of copies of the target genes in the Real-Time RT-PCR reaction.

- Only curves with the typical exponential amplification, meaning the curve of the raw data shows a flat baseline at the beginning, followed by a clear exponential phase and optionally reaching a plateau phase should be regarded positive.

- The actual test analysis starts with the validity check of the entire Real-Time RT-PCR run. Afterwards, by means of the Internal Control the validity of each sample reaction and its true test result can be verified according to the Ct-value of the Internal Control channel (VIC). Finally, the SARS-CoV-2-specifi c status of each sample is analyzed (FAM).

Test Evaluation

- The **Real-Time RT-PCR test run** is only **valid,** if the FAM-curve and VIC-curve of the Negative Control are negative (Ct > 35) and the FAM-curve and VIC-curve of the Positive Control are positive. For a valid test the FAM-Ct-value and VIC Ct-value of the Positive Control have to be > 15 and ≤ 35.

- If one or more of a RNA Isolation Control (RIC(s)) is processed, its FAM- and VIC-curves must be negative.

Target	**Channel**	**Signal**		
Internal Control	VIC	positive	positive / negative	negative
SARS-CoV-2	FAM	negative	positive	negative
The sample is SARS-CoV-2		**negative**	**positive**	**inhibited**

- A **sample is negative for SARS-CoV-2,** if its VIC-curve is positive (Ct ≤ 35), but its FAM-curve is negative.

- A **sample is positive for SARS-CoV-2,** if its FAM-curve is positive (Ct ≤ 42), independent of the VIC-curve.

- A **sample is inhibited,** if neither the FAM-curve nor the VIC-curve are positive.

Recommendation: In the case of an inhibited sample the test may be repeated with a dilution of the RNA preparation at e.g. 1:4. Nuclease Free H2O is used as the diluting agent. Preferably, the entire RNA preparation process is repeated with a new sample using ViroGene RNA/DNA purification products or appropriate alternative.

- Convenient and reliable sample data entry, Real-Time PCR start, final qualitative analysis and documentation can be conducted with the ViroGene® Software, please inquire

5. Product Limitations

- Users must be trained and familiar with this product and procedures prior to application.
- Results generated by this product must be interpreted in context to clinical or further laboratory findings. It is the user's responsibility to verify performance for any procedures applied in their laboratory that are not covered by the performance data shown below.
- A negative result does not confirm status of non-infection, as results depend on appropriate specimen collection, viral load in specimen above LOD and absence of inhibitors, which would lead to invalid results. By use of the Internal Control, this product allows testing for the presence of PCR inhibitors and consequently minimizes the risk of false negative results.

F. Performance Characteristics

1. Analytical Sensitivity

- The Limit of Detection is < 2.0 copies per µl of RNA Lysate using an in-vitro transcribed RNA specific for target gene of SARS-CoV-2.

2. Analytical Specificity

2.1 Inclusivity

- Determination of the epidemiological sensitivity is based on *in silico* testing using the nucleotide sequence database NCBI (National Center for Biotechnology Information). Oligonucleotide sequences (primers and probe) of the method were tested theoretically with regard to a cross-reaction or unspecific hybridization. Therefore, the oligonucleotide sequences were aligned with the non-redundant data bank for DNA and RNA ("GenBank", NCBI) using Primer-BLAST and NBLAST.
- The assay detects all 6292 available full genome sequences of SARS-COV-2.

1.1 Exclusivity

- To address the exclusivity of the S & RdRP-genes detection method, 21 strains found in humans but not belonging to the genus Betacoronavirus lineage B Sarbecovirus have been analyzed using the method. None of the strains were detected by the S & RdRP-genes detection method indicating a very good exclusivity[#].

Strain	Result
HCoV-HKU1	Not detectable
HCoV-OC43	Not detectable
HCoV-NL63	Not detectable
HCoV-229E	Not detectable
MERS-CoV	Not detectable
Influenza A H1N1	Not detectable
Influenza A H3N2	Not detectable
Influenza A H5N1	Not detectable
Influenza A H7N9	Not detectable
Influenza B	Not detectable
Rhinovirus / Enterovirus	Not detectable
Respiratory Syncytial Virus	Not detectable
Parainfluenza Virus 1	Not detectable
Parainfluenza Virus 2	Not detectable
Parainfluenza Virus 3	Not detectable
Parainfluenza Virus 4	Not detectable
Human Metapneumovirus	Not detectable
Adenovirus	Not detectable
Human Bocavirus	Not detectable
Legionella spp.	Not detectable
Mycoplasma spp.	Not detectable

- Furthermore, both assays combined in the ViroGene® SARS-CoV-2 Direct kit were analyzed in-silico for cross-reaction with other viruses using the nucleotide sequence database of the NCBI (National Center for Biotechnology). Therefor the oligonucleotide sequences were tested theoretically with regard to cross-reaction and unspecific hybridization.
- The method does not cross react with other viruses especially not with other Sarbecoviruses such as SARS-CoV or further Coronaviridae and species of orders Alpha-, Beta-, Delta- or Gamma coronavirus`, respectively.

3. Reproducibility

- To test the Intraassay Variance samples with defined CT-values were analyzed in replicates in the same PCR run. The standard deviation of the replicates is < 0.5 CT values, the correlation coefficient is < 5 %.

4. Robustness

- To test the Intraassay Variance samples with defined CT-values were analyzed on different machines using different operators. The standard deviation of the samples is < 0.5 CT values, the correlation coefficient is < 5 %.

5. References

- [#] V. Corman et al., “Diagnostic detection of 2019-nCoV by real-time RT-PCR,” 2020.

G. Symbols used on labels

- In Vitro Diagnostic Medical Device

- Catalogue Number

- Manufacturer