ViroReal® Kit SARS-CoV-2 & SARS

Kit version 1.2

Instructions for Use

CE

IVD **For *in vitro* diagnostic use**

REF	**DHUV02313**	Σ	**100**
REF	**DHUV02313x5**	Σ	**500**
REF	**DHUV02313x50**	Σ	**5000**

ingenetix GmbH
Haidingergasse 1
1030 Vienna, Austria
T +43(0)1 36 198 01
office@ingenetix.com
www.ingenetix.com

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Explanation of symbols

LOT	Batch code		Use by date
REF	Catalogue number		Manufacturer
Σ	Contains sufficient for <n> tests		Temperature limit (Store at)
CE	This product fulfils the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices	IVD	In vitro diagnostic medical device
	Consult instructions for use	UDI	Unique device identifier
	Keep away from sunlight	QTY	Contents

1 Intended purpose

ViroReal® Kit SARS-CoV-2 & SARS is a non-automated IVD test, based on one-step reverse transcription real-time PCR (RT-PCR), for the qualitative detection of RNA of the N gene of SARS-CoV-2, SARS-CoV and SARS-related coronavirus (Sarbecovirus).

Proper specimens are RNA extracts of samples from the human respiratory tract (throat rinsing fluid, nasopharyngeal and oropharyngeal swabs, anterior nasal swab and mid-turbinate nasal swab specimens, nasopharyngeal wash/aspirate and nasal aspirates).
This test is suitable for patients of all ages with or without a suspected SARS-CoV-2 or SARS coronavirus infection and is intended as an aid in the diagnosis of such infections in combination with patient history and additional clinical information.

The test is intended for professional use and is limited to qualified personnel instructed in the procedures of real-time PCR and *in vitro* diagnostic procedures.

2 Product description

ViroReal® Kit SARS-CoV-2 & SARS is a one-step reverse transcription real-time PCR test and detects RNA of the nucleocapsid protein gene (N gene) of SARS-CoV-2 as well as of SARS-CoV and SARS-related coronavirus (Sarbecovirus). Other beta coronaviruses are not detected with this kit.

A probe-specific amplification-curve in the FAM channel indicates the amplification of virus-specific RNA.
The internal RNA positive control (IPC) is detected in Cy5 channel and serves as a control for RNA extraction and possible RT-PCR inhibition. The target for the RNA IPC (artificial target RNA) is added during sample extraction.

The chosen design for the N gene includes a highly conserved region in all SARS coronavirus clusters of the N gene, which allows the universal detection of previously known Sarbecoviruses including SARS-CoV-2 without discriminating between strains. The primer and probe design chosen is not identical with the WHO design. Due to its universal character, it takes into account possible future mutations in the SARS-CoV-2 virus sequence. Because of its design, the test has the potential to detect new SARS corona viruses in the future.

ViroReal® Kit SARS-CoV-2 & SARS was validated with the ABI® 7500 Fast Real-Time PCR System (fast cycle parameters are not supported, Thermo Fisher Scientific), and was also tested with Mx3005P® (Agilent), qTOWER^{3}G (Analytik Jena), MIC instrument (bio molecular systems), LightCycler® 480 I (Roche) and cobas Z 480 Analyzer (Roche). It is also compatible with other real-time PCR instruments which detect and differentiate fluorescence in FAM, and Cy5 channel (eg., QuantStudio™ 5 / 6 / 7 (Thermo Fisher Scientific)). When using PCR-platforms not tested by ingenetix, an evaluation of the multiplex-PCR shall be done. Keep in mind that some PCR-platforms first must be calibrated with the corresponding dye before performing multiplex-PCR.

Ingenetix ViroReal®, BactoReal®, MycoReal®, PanReal and ParoReal® Kits are optimized to run under the same thermal cycling conditions. RNA and DNA can be analysed in one run.

3 Pathogen information

Coronaviruses are positive single-stranded RNA viruses of the family *Coronaviridae*. Several different strains of coronaviruses are currently known to infect humans (HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, MERS-CoV, SARS-CoV, SARS-CoV-2, NCoV and HCoV-EMC).

Strains HCoV-229E, HCoV-NL63, HCoV-OC43, MERS-CoV and HCoV-HKU1 cause cold, upper respiratory infection, bronchiolitis, and pneumonia in humans.

SARS-CoV, a beta coronavirus, causes the Severe Acute Respiratory Syndrome (SARS).

SARS-CoV-2, a beta corona virus, causes COVID-19 (corona virus disease 2019). Fever, cough, and breathing difficulties are described as the most common initial symptoms, and infection can subsequently lead to pneumonia. The coronavirus spreads mainly by droplet and contact transmission.

4 Principle of one-step reverse transcription real-time PCR

The test is based on multiplex one-step reverse transcription real-time PCR (RT-PCR) by 5´-nuclease-assay technology. In a first step, a specific RNA sequence is transcribed into cDNA and subsequently the DNA is amplified. The generated PCR-products are detected by an oligonucleotide-probe labelled with a fluorescent dye. This allows the sequence-specific detection of PCR amplificates.

During PCR, primers are extended by *Taq* polymerase and probes hybridized to the target are cleaved by the 5'-exonuclease activity of *Taq* polymerase. According to the accumulation of PCR product, the fluorescence of the probe increases with each PCR cycle. The change in fluorescence of the different dyes is recorded cycle by cycle in the real-time PCR instrument in the closed reaction tube at different fluorescence wavelengths.
The Cq value (Cq = Quantification cycle, Ct = Cycle threshold, Cp = Crossing point) describes the cycle at which the fluorescence rises significantly above the background fluorescence.

5 Contents of the kit, stability, and storage

Table 1 Order no. DHUV02313

		DHUV02313	
Component	**Content**	**Amount 100 reactions**	**Storage**
SARS-CoV2&SARS + IPC3 Assay Mix (green cap)	Primers and probes for detection of - Sarbecovirus incl. SARS-CoV-2 (FAM) - RNA IPC (Cy5)	1 x 100 µl	-25 to -15 °C
IPC-Target (RNA) (orange cap)	Target for RNA IPC (internal RNA positive control system)	1 x 200 µl	-25 to -15 °C
SARS-CoV-2 Positive Control (red cap)	RNA Positive Control (1000 copies/µl)	1 x 300 µl	-25 to -15 °C
RNA Reaction Mix (white cap)	PCR reaction mix for RNA amplification	1 x 500 µl	-25 to -15 °C
Nuclease-free water (blue cap)	Nuclease-free water	1 x 1000 µl	-25 to -15 °C

Table 2 Order no. DHUV02313x5 and DHUV02313x50

		DHUV02313x5	DHUV02313x50	
Component	**Content**	**Amount 500 reactions**	**Amount 5000 reactions**	**Storage**
SARS-CoV2&SARS + IPC3 Assay Mix (green cap)	Primers and probes for detection of - Sarbecovirus incl. SARS-CoV-2 (FAM) - RNA IPC (Cy5)	1 x 500 µl	1 x 5 ml	-25 to -15 °C
IPC-Target (RNA) (orange cap)	Target for RNA IPC (internal RNA positive control system)	1 x 500 µl	1 x 5 ml	-25 to -15 °C
SARS-CoV-2 Positive Control (red cap)	RNA Positive Control (1000 copies/µl)	1 x 500 µl	1 x 1 ml	-25 to -15 °C
RNA Reaction Mix (white cap)	PCR reaction mix for RNA amplification	5 x 500 µl	1 x 25 ml	-25 to -15 °C
Nuclease-free water (blue cap)	Nuclease-free water	3 x 1000 µl	1 x 25 ml	-25 to -15 °C

RNA Reaction Mix: The Master Mix provided with the kit has been designed for reliable, high-sensitivity one-step reverse transcription real-time PCR even in the presence of common reaction inhibitors. The Master Mix contains a thermostable MMLV Reverse Transcriptase, an RNase inhibitor, a highly purified Taq Polymerase for rapid hot-start PCR, dNTPs, ROX™ dye (passive reference) and buffer components – additives optimized to handle RT-PCR inhibitors.

Delivery and Storage

Shipment is with dry ice. When stored properly, the kit components are stable until the specified expiration date. This also applies after opening. Store kit protected from light.

Quality Control Release Testing

In accordance with the ISO 13485 certified Quality Management System of ingenetix, each lot is tested against predetermined specifications to ensure consistent product quality.

Quality control is performed with an artificial RNA representing parts of the pathogen RNA. The RNA concentration was determined using a standard curve of a plasmid containing parts of the pathogen sequence.

6 Additionally required materials and devices

- Reagents and devices for RNA-extraction which are appropriate for the listed sample material (see 9. Preparation of the samples)
- Nuclease-free water
- Powder-free disposable gloves
- Pipettes (adjustable)
- Filter pipette tips
- Vortex-Mixer
- Benchtop centrifuge with rotor for 2 ml reaction tubes
- Real-time PCR instrument detecting and differentiating fluorescence in FAM and Cy5 channel
- Appropriate 96 well optical reaction plates or reaction tubes with corresponding (optical) closing material
- Optional: Laminar flow box
- Optional: PCR workstation

7 Precautions and safety information

7.1 General information

- For *in vitro* diagnostic use. The use of this kit is limited to qualified personnel instructed in real-time PCR and *in vitro* diagnostic procedures.
- Transportation of clinical specimens must comply with local regulations for the transport of biological materials.
- Specimens should be handled as if infectious in accordance with safe laboratory procedures. Wear protective powder-free disposable gloves when handling kit reagents and specimens.
- Discard specimens, reagents, and waste according to your local safety regulations.
- Improper collection, transport or storage of specimens may hinder the ability of the assay to detect the target sequences.
- Quality of RNA has a profound impact on the test performance. Ensure that the used RNA extraction system is compatible with real-time PCR technology.
- The real-time PCR instrument should be calibrated, serviced, and cleaned regularly.
- Protect kit components from light.
- Avoid mixing reagents of different kits and lots and check expiry date of the kit.

7.2 Specific information

A workflow must be followed to avoid false positive results due to detection of contaminating RNA.

Recommended measures to avoid RNA contamination:

- Use separated workspaces for specimen preparation, set-up of real-time PCR and amplification. Supplies and equipment must be dedicated to each of these separate workspaces to ensure workflow in the laboratory from pre- to post-PCR.
- Laboratory benches and devices must be cleaned regularly.
- Sample preparation should be performed in a laminar flow box. Clean laminar flow box regularly in all areas.
- If possible, leave consumables and pipettes in the laminar flow sterile bench and PCR workstation.
- Preparation of real-time PCR should be done in a PCR workstation.
- The use of sterile aerosol-resistant pipette tips is required.
- Use only RNA-free consumables.
- Wear lab coat.
- Work only with powder-free disposable gloves, do not touch the outer surface of the palm and fingers of the gloves when putting them on. Change gloves frequently. To avoid skin contact, wear gloves over the sleeves of the lab coat. Use disposable sleeve protectors if possible.
- Do not touch the rim or threads of open vials.
- Caution is advised when handling specimens and positive control to avoid cross-contamination.
- Store positive and potentially positive material separately from all other reagents.
- For a valid interpretation of results, a negative control shall be included during RNA-extraction (e.g., extraction of water instead of sample material), to exclude false-positive results due to contamination with RNA of pathogen during extraction.
- Optional: include a negative control of PCR per PCR-run (nuclease-free water instead of sample, NTC).

8 Limitations

- Reliable results with this test are only achieved by appropriate specimen collection, transport and storage, as well as an appropriate RNA extraction procedure.
- With this kit SARS-CoV-2 detection has been validated for swabs from the respiratory tract. Test performance with other specimen types has not yet been assessed.
- A negative test result does not exclude the possibility of a SARS-CoV infection, because test results may be affected by improper specimen collection, technical error, specimen mix-up or viral quantities below the assay sensitivity. The presence of PCR inhibitors may lead to invalid results.

- For this kit highly specific primers and probes have been selected. However, false-negative, or less sensitive results might be obtained due to sequence heterogeneity within the target region of not yet described clinical subtypes.
- This approach allows universal detection of all so far known SARS-CoV strains including SARS-CoV-2 and SARS-like CoV without discriminating between strains.
- Results should be interpreted in context of clinical and laboratory findings.

9 Preparation of samples

ViroReal® Kit SARS-CoV-2&SARS is suitable for analysis of RNA extracts of samples from the human respiratory tract (throat rinsing fluid, nasopharyngeal and oropharyngeal swabs, anterior nasal swab and mid-turbinate nasal swab specimens, nasopharyngeal wash/aspirate and nasal aspirates).

Sample preparation should be performed with the recommended measures to avoid RNA contamination (see precautions and safety information). Always include an extraction negative control during RNA-extraction (e.g., extraction of water instead of sample material).

Purified RNA should be stored at -25 to -15 °C or at -80 to -60 °C.

9.1 Sample collection and storage

Samples from the respiratory tract can be stored in microcentrifuge tubes. Swabs are stored either dry or in isotonic saline solution (NaCl 0.9%, not provided). Swabs can be collected with swab material proper for PCR (e.g., sterile polyester or rayon swabs with aluminium or plastic shaft).
It is recommended to process samples immediately after collection. Store samples at 2-8 °C for no longer than 48 hours. Do not freeze native samples prior to extraction. Samples in lysis buffer can be frozen.

9.2 Recommended extraction methods

Ensure that the extraction system used is not contaminated with RNA of pathogens detected with ViroReal® Kit SARS-CoV-2&SARS. Extract samples with an RNA extraction system compatible with RT real-time PCR technology and appropriate for the sample material. Extract RNA from 140-200 µl sample (depending on the extraction method) and elute in approx. 50 µl.

<u>For manual extraction recommended</u>
- QIAamp Viral RNA Mini Kit (Qiagen)

<u>For automated extraction recommended</u>
- Mag-Bind® Viral RNA XPress KIT (Omega Bio-tek)

When using extraction methods not recommended by ingenetix, an evaluation of the extraction method must be performed.

9.3 Quality control for RNA extraction and RT PCR inhibition with IPC

The RNA IPC system (internal RNA positive control) is used as a control for RNA extraction, identifies possible RT PCR inhibition and confirms the integrity of kit reagents.
For this, an artificial RNA (IPC-Target (RNA), approx. $5x10^5$ copies/µl) is added during extraction.

Note: The Cq values of the IPC depend on the extraction method and on the type of sample material. Negative samples should show Cq values of the IPC between 27-30. The extraction method used must be validated accordingly with sample material. Use the IPC-Target (RNA) freshly diluted (1:10 with nuclease-free water) in the extraction, if Cq values < 27 are determined during validation with samples.

9.3.1 Application of IPC as control of extraction and RT real-time PCR

The IPC-Target (RNA) must be added during extraction.

→ Per sample spike <u>1 µl IPC-Target (RNA)</u> (orange cap) directly into the appropriate volume of lysis buffer (or spike it to the sample <u>after</u> the lysis buffer has been added to the sample), then continue the extraction procedure.

Caution: The IPC-Target (RNA) shall not be added to sample material in the absence of lysis buffer, as degradation may occur. It must be added to the lysis buffer.

9.3.2 Application of IPC as quality control for RT PCR reaction

If the IPC-Target (RNA) has not been added during extraction, it can be added at a later stage to the PCR master mix.

→ Freshly dilute the IPC-Target (RNA) 1:100 with nuclease-free water and add 1 µl of the dilution/PCR reaction.

Caution: The IPC-Target (RNA) shall not be added undiluted to the master mix (inhibition of PCR).

10 Preparation of real-time PCR

- Include one positive control (red cap), one extraction negative control and optionally one PCR negative control (NTC, e.g., nuclease-free water) per PCR run.
- It is generally recommended to analyse samples in duplicates, which increases the probability of pathogen detection and facilitates interpretation of results.
- Best use RNA immediately after extraction and always keep it on ice. Alternatively, use RNA stored at -80 °C to -15 °C. Thaw RNA on ice, avoid prolonged exposure to room temperature and immediately refreeze the RNA after use.
- Just before use, thaw the RNA Reaction Mix, and invert 2 to 3 times to ensure a homogenous solution. The RNA Reaction Mix does not freeze at -20 °C, but gelling may occur. The RNA Reaction Mix should not be warmed to room temperature. Thaw the other kit components completely at room temperature. When thawed, mix components carefully, centrifuge briefly with low speed and subsequently put on ice. Prepare Master Mix for RT-PCR on ice.
- **Positive Control**
  → As positive control, use 10 µl of the Positive Control (red cap). Always, pipette positive control at last. SARS-CoV-2 Positive Control is an *in vitro* synthesized RNA with a concentration of approx. 10^3 copies/µl. It must be stored at -20 °C. Before use, gently mix Positive Control, do not vortex. To avoid frequent freeze/thaw cycles, it can also be temporarily stored at 4 °C if used several times on the same day.

10.1 Pipetting scheme

		Per sample
Preparation of Master Mix (mix well)	Nuclease-free Water*	4.0 µl
	RNA Reaction Mix	5.0 µl
	SARS-CoV-2 & SARS + IPC3 Assay Mix	1.0 µl
	Total volume Master Mix	**10.0 µl**
Preparation of RT-PCR	Master Mix	10.0 µl
	RNA-Sample*	10.0 µl
	Total volume	**20.0 µl**

*10 µl sample can be used. When using a volume other than 10 µl, the volume of nuclease free water has to be adjusted accordingly.

→ **If IPC-Target (RNA) was not added during extraction:** Freshly dilute the IPC-Target (RNA) (orange cap) 1:100 with nuclease-free water and add 1 µl per sample directly to the master mix. In this case, the IPC-Target (RNA) is used as a quality control for the RT PCR reaction.

Caution: The use of more than 1 µl diluted IPC-Target (RNA) per reaction might cause inhibition of the real-time PCR reaction.

- Prepare the Master Mix according to the number of samples, calculating an additional volume of approx. 10% for pipetting loss.
- Pipette 10 µl of the prepared Master Mix per sample into the well of the optical reaction plate.
- Then add 10 µl of the extracted sample or controls. Pipette the positive control at last.
- Seal the plate with a suitable optical sealing material.
- Vortex the sealed plate for 1-2 seconds and briefly centrifuge the plate.

10.2 Programming of temperature profile

Please find further information on programming of the real-time PCR instrument in the respective operator's manual. Keep in mind that some PCR-platforms must be calibrated with the corresponding dye before performing a multiplex-PCR.

Sample Volume: 20 µl
Ramp speed: "Standard" without "fast cycling" parameter for ABI® 7500 Fast Instrument, QuantStudio™ (Thermo Fisher Scientific)

Program 1 Cycles: 1 Analysis: None	**Program 2** Cycles: 1 Analysis: None	**Program 3** Cycles: 45 Analysis: Quantification Acquisition at 60 °C
50 °C 15 min	95 °C 2 min	95 °C 5 sec 60 °C 1 min

Ad program 1: This temperature profile can be used for all ingenetix ViroReal®, BactoReal®, MycoReal®, PanReal and ParoReal® kits for the detection of DNA or RNA.
Ad program 2: the previous temperature profile with 20 sec in program 2 can still be used.

<u>Select detection channels</u>

FAM channel: Detection of SARS-CoV-2 or SARS-CoV
Cy5 channel: Detection of IPC

For ABI® 7500 Instrument, QuantStudio™ 5/6/7 (Thermo Fisher Scientific), Mx3005P® (Agilent)
FAM-TAMRA
Cy5-NONE
Passive reference dye: ROX

For MIC Instrument (bio molecular systems)
FAM: Green
Cy5: Red
Passive reference dye: no ROX needed

For cobas z 480 Analyzer / LightCycler® 480 II (Roche)
Detection format: 2 Color Hydrolysis Probe
FAM: Excitation at 465 nm, Emission at 510 nm
Cy5: Excitation at 610 / 618 nm, Emission at 670 / 660 nm
Passive reference dye: no ROX needed

11 Interpretation of data

For the analysis of the PCR results, select the fluorescence display options FAM channel for the pathogen target and Cy5 channel for the IPC.
Please note that some PCR platforms require Color Compensation when using a multiplex PCR.

IMPORTANT: Samples should be checked in both logarithmic (Roche instrument: Abs Quant/Fit Points) and linear view and compared to the negative control. In addition to the Cq values, also check the amplification curves and adjust the threshold (noise band) if necessary.
After you have saved the new settings, export the data. For the cobas z 480 Analyzer, export tables per dye.

Assessment of clinical specimen test results should be performed after data of the positive and negative controls and of the IPC have been examined and determined to be valid. If results of controls are not valid, no interpretation of results with clinical samples is possible.

Negative samples:
Samples without amplification curves (no Cq values, undetermined) are regarded as negative. No RNA was detected in these samples because there is no infection with the corresponding pathogen, or the pathogen RNA concentration is below the detection limit of the test.

Positive samples:
Samples with Cq values < 45 in the fluorescence channel for the pathogen target are regarded as positive.

Table 3 shows the criteria for valid positive and negative controls. Table 4 shows interpretation of data with clinical samples.

11.1 Controls

Table 3 Criteria for valid positive and negative controls, IPC-Target (RNA) was added during extraction

	Cq FAM channel SARS target	Cq Cy5 channel IPC[1]	Interpretation	Action
Positive control	<30	Negative	Valid	-
Positive control	Negative	Negative	Invalid	See 12.1
Extraction negative control	Negative	27-30[2]	Valid	-
Extraction negative control	Negative	Negative	Invalid	See 12.1
Extraction negative control	Positive	27-30[2]	Invalid	See 12.3
NTC PCR (optional)	Negative	Negative	Valid	-
NTC PCR (optional)	Positive	Negative	Invalid	See 12.2

[1] If the IPC-Target (RNA) was added directly to the master mix, all samples in the Cy5 channel must be positive.
[2] Cq values of the IPC are dependent on the extraction method and should show Cq values of 27-30 (see 9. preparation of samples).

11.2 Clinical samples

Samples with positive Cq-values are considered positive, see Table 4.

Table 4 Interpretation of data with clinical samples

	Cq FAM channel SARS target	Cq Cy5 channel IPC[1]	Interpretation	Action
Clinical sample	Negative	27-30[2]	Negative	-
Clinical sample	Positive	Positive/Negative[2]	Positive	-
Clinical sample	Negative	Negative	Invalid	See 12.5

[1] A positive signal of the IPC excludes potential PCR inhibition. However, IPC Cq-values should show comparable results among samples. A shift of Cq- values can indicate a partial inhibition of PCR. Cq values of the IPC are dependent on the extraction method and should show Cq values of 27-30 (see 9. preparation of samples).
[2] High pathogen load in the sample can lead to a reduced or absent fluorescence signal of the IPC.

In case of invalid data, analysis has to be repeated with the remaining or newly extracted RNA sample (see 12. Troubleshooting).

12 Troubleshooting

12.1 No virus specific signal with positive control and with IPC

- Incorrect programming of the temperature profile or incorrect setting of detection channels on the real-time PCR instrument.
  → Compare temperature profile and setting of detection channels with details specified in the protocol.
- Incorrect configuration of the PCR reaction.
  → Check your pipetting steps with the pipetting scheme and repeat PCR, if necessary.
- The RNA may have been degraded.
- The IPC-Target (RNA) was added undiluted directly to the mastermix and not freshly diluted 1:100. The PCR reaction is therefore inhibited.
  → Freshly dilute IPC-Target (RNA) 1:100 and repeat PCR.
- No Positive Control was added.
  → Repeat PCR in case all clinical samples are negative.
- For control of the reverse transcription real-time PCR only, 1 µl of freshly 1:100 diluted IPC-Target (RNA) must be added to the mastermix. If the IPC-Target (RNA) has been forgotten to be added:
  → Freshly dilute IPC-Target (RNA) and repeat PCR.
- For control of RNA extraction and PCR inhibition, the undiluted IPC-Target (RNA) must be added during extraction to the lysis buffer. If the IPC-Target (RNA) has been forgotten to be added:
  → Repeat RNA extraction.

12.2 Virus specific signal with negative control

- A contamination occurred during preparation of the RT-PCR.
  → Repeat the RT-PCR with new reagents in replicates.
  → Strictly pipette positive control at last.
  → Make sure that workspace and instruments are cleaned at regular intervals.

12.3 Virus specific signal with negative control of extraction

- A contamination occurred during extraction.
  → Repeat RNA extraction and RT-PCR using new reagents.
  → Make sure that workspace and instruments are cleaned at regular intervals.

12.4 IPC specific signal with PCR negative control and positive control

- The IPC-Target (RNA) has been added to lysis buffer during extraction, but there is IPC specific signal with PCR negative control and positive control: Contamination with the IPC-Target (RNA).
  → Make sure that workspace and instruments are cleaned at regular intervals.

12.5 No signal with IPC and no virus specific signal with sample

- Incorrect assignment of detection channels in sample.
  → Please verify the correct assignment of detection channels.
- Incorrect RT-PCR conditions.
  → Check the RT-PCR conditions and repeat the RT-PCR, if necessary.
- The RNA might be degraded.
- If IPC-Target (RNA) was added during extraction:
  - Inhibition of RT-PCR may have occurred.
  - RNA extraction was unsuccessful.
  - The IPC-Target (RNA) was not added to the lysis buffer of the sample.
  - The extracted sample was not added to the RT-PCR reaction.

  → No statement is possible. Verify you use a recommended method for RNA isolation and re-examine the single steps of the RNA extraction.
- → If no operating mistakes during RNA extractions can be retraced, it is recommended to repeat the RT-PCR with lower amounts of RNA-eluate (1/5 or 1/10 of sample volume + the adequate volume of nuclease-free water).

13 Specifications and performance evaluation

13.1 Test performance on different real-time PCR instruments

Performance of ViroReal® Kit SARS-CoV-2 & SARS with an ABI® 7500 instrument is shown in Figure 2.

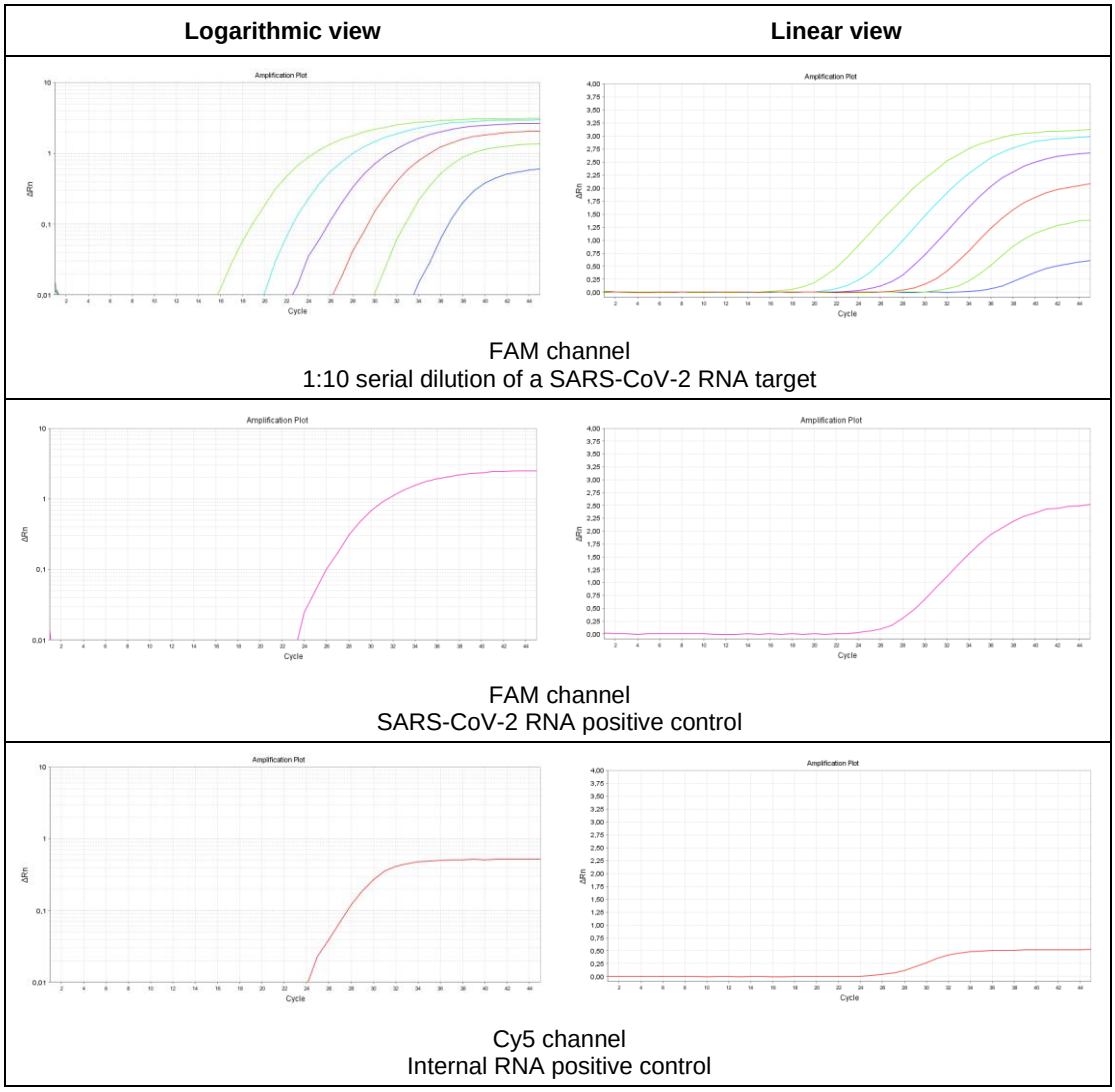

Figure 2 Performance of ViroReal® Kit SARS-CoV-2 & SARS

ViroReal® Kit SARS-CoV-2 & SARS was validated with the ABI® 7500 Fast Real-Time PCR System (fast cycle parameters are not supported, Thermo Fisher Scientific), and was also tested with Mx3005P® (Agilent), qTOWER³G (Analytik Jena), MIC instrument (bio molecular systems), LightCycler® 480 I (Roche) and cobas Z 480 Analyzer (Roche).

Compatibility of ViroReal® Kit SARS-CoV-2 & SARS was validated with QIAamp Viral RNA Mini Kit (Qiagen) and Mag-Bind® Viral RNA XPress KIT (Omega Bio-tek)

13.2 Limit of detection, LoD95%

Method: To determine the LoD95% (LoD95% = smallest number of copies of target RNA which could be detected in 95% of 20 extractions), a commercially available SARS-CoV-2 RNA (AMPLIRUN® Coronavirus SARS-CoV-2 RNA control, Vircell, order no. MBC137-R) was serially diluted in sputum (1:1 diluted with Sputasol) and extracted. Extractions were performed with the QIAamp Viral RNA Mini Kit (140 µl sputum extracted, RNA eluted in 60 µl) and real-time PCR was performed on ABI® 7500 instrument. The LoD95% was determined by extracting and testing different concentrations in 20 replicates.
Result: The LoD95% was 893 copies/ml sputum, which corresponds to approx. 21 target copies/PCR reaction.

13.3 Linearity and dynamic measuring range

Method: Linearity was determined using a 10-fold dilution series (10^6 – 10^1 target copies/reaction) of the synthetic RNA. The number of determinations (n) per dilution was nine.

Result: The assay shows linearity over the range of 10 to 1,000,000 target copies/ with a slope of -3.28 and an R^2 of 0.99 as shown in Figure 3.

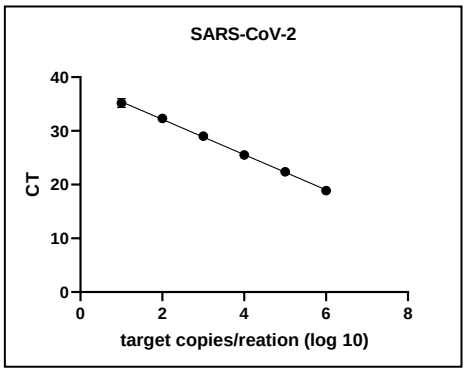

Figure 3 Ten-fold dilution series of a SARS-CoV-2 RNA standard plotted against Ct

13.4 Precision inter-assay

Method: Inter-assay precision is defined as the reproducibility of a sample between assay runs. The inter-assay precision of ViroReal® Kit SARS-CoV-2 & SARS was determined from 10-fold dilutions of an RNA (1.00E+06 to 1.00E+00 target copies/reaction) in three independent experiments performed on different days in quadruplicates.

Result: Precision standard replicate coefficient of variation (CV%) ranged from 0.3% to 1.88%, with mean overall inter-assay precision equal to 0.85%.

13.5 Precision intra-assay

Method: Intra-assay precision is defined as the reproducibility of a sample within an assay run. Intra-assay precision of ViroReal® Kit SARS-CoV-2 & SARS was determined from the replicate runs above.

Result: CV% ranged from 0.33% to 2.90%, with mean overall intra- assay precision equal to 0.97%.

13.6 Analytical specificity

Method: Specificity is ensured by the selection of highly specific primers and probes. A highly conserved region in all SARS coronavirus clusters of the N gene was chosen as target region. The selected primer and probes should cover possible future changes in the virus sequence and are therefore not identical with the primer and probes suggested by the WHO.
In silico validation of primers and probes was carried out with the Basic Local Alignment Search Tool (BLAST) against the NCBI database and with comparative sequence analyses in the GISAID database. All the data in the two databases showed homology in the sequence region of primer and probes throughout all SARS coronavirus clusters. This approach allows specific and universal detection of all so far known SARS coronavirus strains including SARS-CoV-2 without discriminating between strains.

Result: ViroReal® Kit SARS-CoV-2 & SARS detects specifically the N gene of SARS-CoV-2 as well as SARS-CoV and SARS-related bat coronavirus. Other beta coronaviruses are not detected with this kit.

13.7 Diagnostic evaluation

Method: ViroReal® Kit SARS-CoV-2 & SARS was tested by an external service provider (AGES, Vienna) on 94 swab samples from the respiratory tract collected from patients suspected to suffer from COVID-19. Extraction was performed with the MagNA Pure Compact using the Total Nucleic Acid Isolation Kit (Roche). Analyses were performed in single reactions on the LightCycler® 480 I instrument (Roche).
In house test (reference method): results were compared with results of a real-time PCR test detecting the E gene of SARS-CoV-2, according to the recommendations of the WHO: Detection of 2019 novel coronavirus (2019-nCoV) (Victor M Corman et al., 2020).

Result: Fifteen out of the 94 samples were positive with both methods. One sample was positive with ViroReal® Kit SARS-CoV-2 & SARS only. Due to the high Cp-value, this sample is considered as a true positive one, with a low concentration of SARS-CoV-2 RNA in the range around the detection limit, which might explain the negative test result with the reference method. The remaining samples were negative with both methods, see Table 5, Table 6.

Table 5 Diagnostic evaluation of ViroReal® Kit SARS-CoV-2 & SARS

	Reference			
		pos	**neg**	**Total**
ViroReal® Kit SARS-CoV-2	**pos**	**15**	**1***	**16**
	neg	**0**	**78**	**78**
	Total	**15**	**79**	**94**

*Weak positive, Cp-value = 35.5

Table 6 Diagnostic performance

	Value	95% CI
Sensitivity	100 %	79.41% to 100.00%
Specificity	100 %	95.38% to 100.00%
NPV	100 %	100 %
PPV	100 %	100 %
Prevalence	17.02 %	10.05% to 26.16%

14 References

Huang, Chaolin & Wang, Yeming & Li, Xingwang & Ren, Lili & Zhao, Jianping & Hu, Yi & Zhang, Li & Fan, Guohui & Xu, Jiuyang & Gu, Xiaoying & Cheng, Zhenshun & Yu, Ting & Xia, Jiaan & Wei, Yuan & Wu, Wenjuan & Xie, Xuelei & Yin, Wen & Li, Hui & Liu, Min & Cao, Bin. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet. 10.1016/S0140-6736(20)30183-5.
https://www.who.int/emergencies/diseases/novel-coronavirus-2019

15 Revision history

Revision	Date	Description
1.1en	20 March 2020	
1.2en	06 April 2020	New kit version 1.1 launched for order numbers DHUV02313 (100 reactions) and DHUV02313x5 (500 reactions). Changes only affect the filling and number of reaction tubes, the test performance itself has not been changed. This concerns lots produced after 03 April 2020. 4.2. Kit with order number DHUV02353 (50 reactions) is no longer available. New order number DHUV02313x50 (5,000 reactions) available. Kit with order number DHUV02313 (100 reactions) version 1.1: The SARS-CoV-SARS Assay Mix and the RNA IPC-3 Assay Mix have been combined in one assay mix (100 µl, green lid). The RNA Reaction Mix (500 µl) is filled in one 0.5 ml tube. Kit with order number DHUV02313x5 (500 reactions) Version 1.1: The SARS-CoV-2 & SARS + RNA IPC-3 Assay Mix (500 µl) is filled in one 0.5 ml tube 8.1. Pipetting scheme changed

		10.2. Troubleshooting: Information added that in case of a background noise increase in the NTC the threshold has to be adjusted The appendix contains information on the kit contents and pipetting scheme of the previous version.
1.3en	13 August 2020	1. Updated information on intended use 4.2. DHUV02313x5: SARS-CoV-2 Positive Control changed from 120 µl to 300 µl 5. Updated information on validated real-time PCR instruments and extraction kits 7. Updated information on limitations 8. Updated information on sample collection 8.4. Information on detection channel for Mic instrument added 9. Updated information on interpretation of data 10. Updated information on troubleshooting 11.1. Updated information on performance of the kit on different real-time PCR instruments 11.2.1. Updated information on LoD per ml extracted sputum Appendix removed
1.4en	03 September 2020	1. Throat rinsing fluid was added 8. Negative control of RNA extraction must always be included 9. Table 1 is corrected, Table 2 is removed 10.2 Figure 1 is removed
1.5en	01 May 2023	**Change of the company address** **Explanation of symbols** Update **5. Contents, stability and storage** Kit version 1.2: Changed filling volume of DNA IPC Target and Positive Control in DHUV02313 Changed filling volume of Positive Control in DHUV02313x5 The RNA IPC-Target is not stored in RNA/DNA Stabilizer anymore and was renamed to IPC-Target (RNA). SARS-CoV-2&SARS+RNAIPC Assay Mix was renamed to SARS-CoV-2&SARS + IPC3 Assay Mix **6. Additionally required materials and equipment** Update **7. Precautions and safety instructions** Update of this section Inclusion of additional information on specimen collection and storage, processing of specimens after collection, storage conditions of specimens, transport and storage conditions of purified RNA. Recommended measures to avoid RNA contamination added. **9. Preparation of samples** Update Note that for Cq values <27, the IPC-Target (RNA) must be diluted prior to extraction. **10. Preparation of real-time PCR** The pipetting scheme has changed. Dilution of IPC-Target (RNA) was changed from 1:500 to 1:100 The temperature profile has changed - the activation step in program 2 has been extended from 20 sec to 2 min. **11. Interpretation of PCR data** Update of tables for results with positive and negative controls and clinical samples. Inclusion of an explanation of how to evaluate clinical specimen test results in relation to controls. **12. Troubleshooting** Update regarding references in the new results tables. **13. Specifications and performance evaluation** Update.

Note:
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Technical support
ingenetix GmbH, Haidingergasse 1, 1030 Vienna, Austria
Telephone: +43 (0)1 36 198 01; **E-Mail:** office@ingenetix.com