

ViroReal[®] Kit Human Coronavirus alpha

Manual



For Research Use Only



DHUV01153



50 reactions



ingenetix GmbH
Arsenalstraße 11
1030 Vienna, Austria
T +43 (0)1 36 198 0 198
F +43 (0)1 36 198 0 199
office@ingenetix.com
www.ingenetix.com

Index

| | |
|--|---|
| 1. Intended use..... | 3 |
| 2. Product description..... | 3 |
| 3. Pathogen information..... | 3 |
| 4. Contents of the kit, stability and storage..... | 4 |
| 5. Additionally required materials and devices..... | 4 |
| 6. Precautions and safety information..... | 4 |
| 7. Limitations..... | 5 |
| 8. Preparation of samples..... | 5 |
| 9. Preparation of real-time PCR..... | 5 |
| 9.1. Pipetting scheme..... | 5 |
| 9.2. Programming of temperature profile..... | 6 |
| 10. Interpretation of PCR-data..... | 6 |
| 11. Troubleshooting..... | 7 |
| 11.1. No signal in FAM, VIC/HEX and Cy5 channel with controls and sample..... | 7 |
| 11.2. Valid results for controls, but no signal in FAM, VIC/HEX and Cy5 channel with sample..... | 7 |
| 11.3. Signal in FAM and/or VIC/HEX channel in negative control..... | 7 |
| 11.4. Signal in FAM and/or VIC/HEX channel in extraction negative control..... | 7 |
| 12. Specifications and performance evaluation..... | 8 |
| 12.1. Kit performance..... | 8 |
| 12.2. Analytical specificity..... | 8 |
| 13. References..... | 8 |

Explanation of symbols



Batch code



Catalogue number



Contains sufficient for <n> tests



Corrosion, GHS05



Use by



Manufactured by



Store at



Exclamation mark, GHS07

1. Intended use

ViroReal® Kit Human Coronavirus alpha is a real-time PCR assay for detection of RNA of human coronavirus NL63 and 229E using multiplex reverse transcription real-time PCR.

2. Product description

ViroReal® Kit Human Coronavirus alpha detects the N gene of human coronavirus (HCoV) NL63 and 229E. This test allows the rapid and sensitive detection and differentiation of RNA of HCoV-NL63 and HCoV-229E from samples purified from specimen of the respiratory tract (e.g. with the QIAamp Viral RNA Mini Kit, Qiagen).

HCoV-NL63 is detected in FAM channel (530 nm), HCoV-229E is detected in VIC/HEX channel (554 nm). An internal RNA positive control (RNA IPC) is detected in Cy5 channel and is used as RNA extraction as well as real-time PCR inhibition control. The target for the RNA IPC is extracted with the sample.

This test has been validated with the Applied Biosystems® 7500 Fast Real-time PCR System (Thermo Fisher Scientific) and tested with a LightCycler® 480 Instrument II (Roche) and Mx3005P® QPCR System (Agilent), but is also compatible with other real-time PCR instruments which detect and differentiate fluorescence in FAM and Cy5 channel.

The test is based on one-step reverse transcription real-time PCR. A specific RNA sequence of the pathogen genome is transcribed to cDNA and amplified. The generated PCR-product is detected by an oligonucleotide-probe labelled with a fluorescent dye. This technology allows a sequence-specific detection of PCR amplicates.

BactoReal®, ParoReal and ViroReal® 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 (+)ssRNA 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, 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 causes the Severe Acute Respiratory Syndrome.

4. Contents of the kit, stability and storage

| Labelling | Content | Amount | Storage |
|---------------------------------------|--|-------------|------------------|
| HCoV alpha Assay Mix (green cap) | Primer and probe (FAM and VIC/HEX) for detection of HCoV NL63 and 229E | 1 x 50 µl | -15 °C to -25 °C |
| RNA IPC-3 Assay Mix (yellow cap) | Primer and probe (Cy5) for RNA IPC detection | 1 x 50 µl | -15 °C to -25 °C |
| RNA IPC Target (orange cap) | Target for RNA IPC (internal RNA positive control system) | 1 x 100 µl | -15 °C to -25 °C |
| HCoV alpha Positive Control (red cap) | RNA positive control for HCoV-NL63 and HCoV-229E | 1 x 150 µl | -15 °C to -25 °C |
| RNA Reaction Mix (white cap) | RNA amplification mix | 1 x 250 µl | -15 °C to -25 °C |
| Nuclease-free water (blue cap) | Nuclease-free water | 1 x 1000 µl | -15 °C to -25 °C |

The components of ViroReal® Kit Human Coronavirus alpha are stable until the expiry date stated on the label.

5. Additionally required materials and devices

- Reagents and devices for RNA-extraction
- Nuclease-free water for dilution of RNA IPC Target and positive control
- Disposable powder-free gloves
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- Real-time PCR instrument which is able to detect and differentiate fluorescence in FAM and Cy5 channel
- Optical 96 well reaction plates or optical reaction tubes

6. Precautions and safety information

- Clean benches and devices periodically.
- Use sterile filter pipette tips.
- Specimens should be handled as if infectious in accordance with safe laboratory procedures. Wear protective disposable powder-free gloves when handling kit reagents and specimens.
- Use separated working areas for specimen preparation, reagent preparation and amplification. Supplies and equipment must be dedicated to each of these separate working areas and ensure workflow in the laboratory from pre- to post-PCR.
- Be careful when handling samples and positive control to avoid cross contamination. Change gloves after handling of samples or positive control. Store positive or potentially positive material separately from reagents
- Prevent contamination of work equipment and reagents with DNA/RNA, nuclease or amplification products by good laboratory practice.
- Quality of RNA has a profound impact on the test performance. Ensure that the used RNA extraction system is compatible with one-step reverse transcription real-time PCR technology.
- Always include a negative control per PCR-run (nuclease-free water instead of sample).
- For a valid interpretation of results, a negative control should be included during RNA-extraction (e.g. extraction of nuclease-free water instead of sample material), in order to exclude false-positive results due to contamination with RNA of pathogen during extraction.
- Please note the expiry date of the kit.
- Do not interchange or mix reagents from kits with different lot numbers.
- Repeated thawing and freezing of kit components should be avoided. Protect kit components from light.
Caution: The RNA IPC Target is stored in RNA/DNA stabilizer which contains Guanidinium thiocyanate/Triton X-100 (see MSDS, www.ingenetix.com).
- Use established laboratory practices according to your local safety regulations for discarding specimens, reagents and waste.

7. Limitations

- Optimal performance of this test requires appropriate specimen collection, transport and storage, as well as an appropriate RNA extraction procedure.
- Test results may be affected by improper specimen collection, technical error, specimen mix-up or pathogen 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.

8. Preparation of samples

Extract samples with an RNA extraction system compatible with reverse transcription real-time PCR technology. An extraction negative control should be included during RNA-extraction (e.g. extraction of water instead of sample material).

The **RNA IPC Target** has to be added during extraction. The RNA IPC is used as a control of RNA extraction, identifies possible PCR inhibition and confirms the integrity of kit reagents.

Caution: The RNA IPC Target must not be added directly to the sample material but has to be pipetted to the lysis buffer.

→ For an elution volume of 50-100 µl: Per sample, spike 1 µl RNA IPC Target into lysis buffer.

→ For an elution volume of >100 µl or when using an automated extraction system: Per sample, spike 2 µl RNA IPC Target into lysis buffer.

9. Preparation of real-time PCR

- Include one negative control (water), one positive control and one extraction negative control per PCR run.
- It is recommended to analyse samples in duplicates, which increases the probability of pathogen detection and facilitates interpretation of results.
- Thaw RNA samples on ice.
- Thaw kit components at room temperature. When thawed, mix components, centrifuge briefly and keep on ice.
- Mix the RNA Reaction Mix to ensure homogeneity of solution.

HCoV alpha Positive Control is an *in vitro* synthesized RNA containing fragments of HCoV-NL63 and HCoV-229E with a concentration of approx. 10^3 copies/µl. It has to be stored at -20°C. Ensure a homogenous solution by gently mixing, do not vortex. To avoid freeze/thaw cycles, it can also be temporarily stored at 4°C if used several times on the same day.

→ As positive control please use 10 µl of the Positive Control.

9.1. Pipetting scheme

| | | Per sample |
|--|--------------------------------|-------------------|
| Preparation of Master Mix (mix well) | Nuclease-free Water* | 3.0 µl |
| | RNA Reaction Mix | 5.0 µl |
| | HCoV alpha Assay Mix | 1.0 µl |
| | RNA IPC-3 Assay Mix | 1.0 µl |
| | Total volume Master Mix | 10.0 µl |
| Preparation of PCR | Master Mix | 10.0 µl |
| | RNA-Sample* | 10.0 µl |
| | Total volume | 20.0 µl |

*1-10 µl of the sample can be used. When using < 10 µl sample, the volume of water has to be adjusted accordingly.

→ **If RNA IPC Target was not added during extraction:** Freshly dilute the RNA IPC Target 1:500 with nuclease-free water and add 1 µl per sample directly to the master mix.

Caution: The use of more than 1 µl diluted (1:500) RNA IPC Target per reaction causes inhibition of the real-time PCR reaction.

9.2. Programming of temperature profile

Please find further information on programming the real-time PCR instrument in the respective operator's manual. Take into consideration that some PCR-platforms have to be calibrated with the corresponding dye before performing a multiplex-PCR.

Select detection channel: FAM-TAMRA, 530 nm (for HCoV-NL63)
VIC/HEX-TAMRA, 554 nm (for HCoV-229E)
Cy5-NONE, 667 nm (for RNA IPC-3)

Select reference dye (passive reference): ROX

Sample Volume: 20 µl

Temperature Profile:

| Program 1 | Program 2 | Program 3 |
|-----------------------------|-----------------------------|--|
| Cycles: 1 Analysis: None | Cycles: 1 Analysis: None | Cycles: 45 Analysis: Quantification Acquisition at 60° |
| 50°C 15 min | 95°C 20 sec | 95°C 5 sec 60°C 1 min |

For ABI PRISM® 7500:
Ramp speed: "Standard"

For LightCycler® 480 instrument:
Detection format: 3 Color Hydrolysis Probe

Note: These parameters are valid for all BactoReal®, MycoReal, ParoReal, and ViroReal® kits.

10. Interpretation of PCR-data

For analysis of PCR results select fluorescence display options 530 nm (FAM channel) for the HCoV-NL63 target, 554 nm (VIC/HEX) for detection of HCoV-229E target and 667 nm (Cy5 channel) for the RNA IPC target. Samples with positive Ct or Cp-values are considered positive. Please additionally check the amplification-curves manually. Samples should be inspected both in logarithmic and linear scale view and compared with the negative control.

| | FAM channel HCoV-NL63 target | VIC/HEX channel HCoV-229E target | Cy5 channel RNA IPC target | Interpretation |
|-----------------------------|---------------------------------|-------------------------------------|----------------------------------|-----------------------|
| Negative control | Negative | Negative | Negative / Positive ¹ | Valid |
| Positive control | Positive | Positive | Negative / Positive ¹ | Valid |
| Extraction negative control | Negative | Negative | Positive | Valid |
| Sample | Negative | Negative | Positive | Negative ³ |
| Sample | Negative | Negative | Negative | Invalid |
| Sample | Positive | Positive | Positive / Negative ² | Positive |
| Sample | Positive | Negative | Positive / Negative ² | Positive |
| Sample | Negative | Positive | Positive / Negative ² | Positive |

¹Only positive if the RNA IPC Target was added at a 1:500 dilution directly to the master mix

²High pathogen load in the sample can lead to reduced or absent signal of RNA IPC

³The positive signal of the RNA IPC excludes a possible PCR inhibition. However, IPC Ct-values should show comparable results. A shift of Ct-values can indicate a partial inhibition of PCR.

In case of invalid data, analysis has to be repeated with the remaining or newly extracted RNA sample (see 11. Troubleshooting).

11. Troubleshooting

11.1. No signal in FAM, VIC/HEX and Cy5 channel with controls and sample

- Incorrect programming of the temperature profile or detection channels of the real-time PCR instrument.
→ Compare temperature profile and programming of detection channels with the protocol.
- Incorrect configuration of PCR reaction.
→ Check your work steps (see pipetting scheme) and repeat PCR, if necessary.

11.2. Valid results for controls, but no signal in FAM, VIC/HEX and Cy5 channel with sample

- Incorrect programming of detection channels with the sample.
→ Compare programming of detection channels with protocol.
- If the RNA IPC Target was added during extraction:
 - PCR reaction has been inhibited.
 - RNA extraction has failed.
 - The undiluted RNA IPC Target has not been added to lysis buffer of sample.
 - The extracted sample has not been added to PCR reaction.
→ No interpretation can be made. Make sure you use a recommended method for RNA isolation and stick closely to the manufacturer's instructions. Check your work steps.

11.3. Signal in FAM and/or VIC/HEX channel in negative control

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

11.4. Signal in FAM and/or VIC/HEX channel in extraction negative control

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

12. Specifications and performance evaluation

12.1. Kit performance

Performance of ViroReal® Kit Human Coronavirus alpha with an Applied Biosystems® 7500 Fast Real-time PCR System (Thermo Fisher Scientific) is shown in Figure 1.

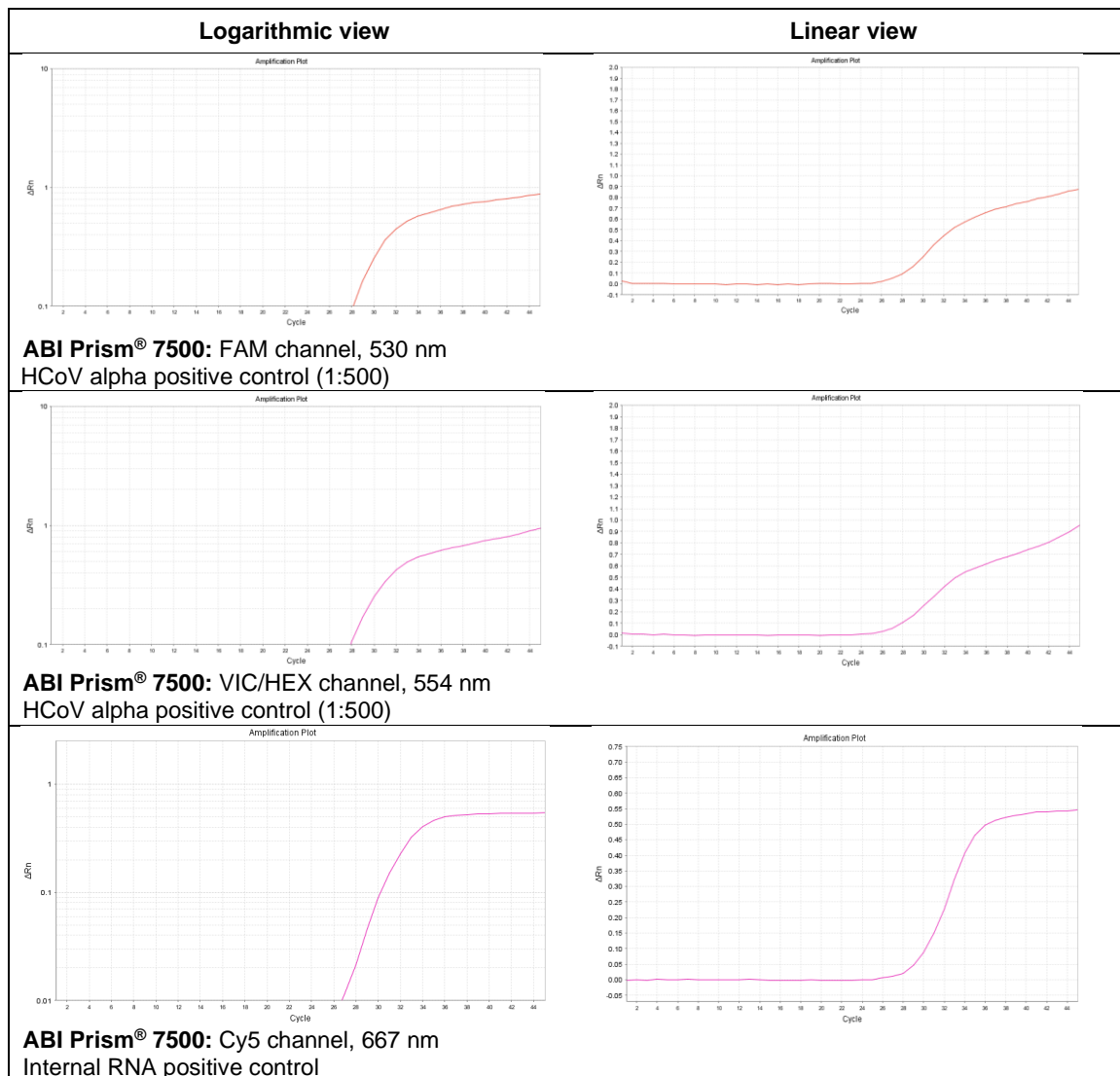


Figure 1 Performance of ViroReal® Kit Human Coronavirus alpha

12.2. Analytical specificity

The selection of highly specific primers and probes ensures analytical specificity. Primers and probes have been checked for possible homologies to currently published sequences by sequence comparison analysis. This also validated the detection of so far known coronavirus alpha strains.

13. References

Walsh EE, Shin JH, Falsey AR. 2013. Clinical impact of human coronaviruses 229E and OC43 infection in diverse adult populations. J Infect Dis; 208:1634.