

# ViroReal<sup>®</sup> Kit Parechovirus

## Manual



For Research Use Only

**REF** DHUV01753

**Σ** 50 reactions



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## 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 Parechovirus is a real-time PCR assay for detection of RNA of human parechovirus strains 1-6 using reverse transcription real-time PCR.

## 2. Product description

ViroReal® Kit Parechovirus detects the polyprotein gene of human parechovirus. This test allows the rapid and sensitive detection of RNA of parechovirus samples purified from serum, cerebrospinal fluid, nasal discharge, urine or stool (e.g. with the QIAamp Viral RNA Mini Kit, Qiagen).

A probe-specific amplification-curve in the FAM channel indicates the amplification of parechovirus specific RNA. 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

Human parechoviruses are non-enveloped, positive single-stranded RNA viruses (ss(+)RNA) and are members of the *Picornaviridae* family. Six serotypes of human parechovirus have been identified (human parechovirus 1-6). Parechoviruses are responsible for gastrointestinal, respiratory and central nervous system infections. 95% of humans are carriers of human parechovirus since early life.

## 4. Contents of the kit, stability and storage

Labelling	Content	Amount	Storage
Parechovirus Assay Mix (green cap)	Primer and probe (FAM) for parechovirus-detection	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
Parechovirus Positive Control (red cap)	RNA positive control (approx. 500,000 target copies/µl)	1 x 15 µl	-15 °C to -25 °C
RNA Reaction Mix (white cap)	4 x RNA reaction 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 Parechovirus 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:** Positive Control and RNA IPC Target are stored in RNA/DNA stabilizer which contains Guanidinium thiocyanate/Triton X-100 (see MSDS, [www.ingenetix.com](http://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.
  - **Parechovirus Positive Control** is an *in vitro* synthesized fragment of parechovirus RNA in RNA-stabilizer. Before use, freshly dilute positive control 1:500 with nuclease-free water.
    - Use 1 µl of freshly 1:500 diluted Parechovirus Positive Control + 9 µl nuclease-free water.
- Caution:** The use of more than 1 µl diluted (1:500) positive control per reaction causes inhibition of the RT-PCR reaction. Pipette positive control at last.

### 9.1. Pipetting scheme

		<b>Per sample</b>
<b>Preparation of Master Mix</b> (mix well)	Nuclease-free Water*	3.0 µl
	RNA Reaction Mix	5.0 µl
	Parechovirus Assay Mix	1.0 µl
	RNA IPC-3 Assay Mix	1.0 µl
	<b>Total volume Master Mix</b>	<b>10.0 µl</b>
<b>Preparation of PCR</b>	Master Mix	10.0 µl
	RNA-Sample*	10.0 µl
	<b>Total volume</b>	<b>20.0 µl</b>

\*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 parechovirus)  
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: 2 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 parechovirus 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 Parechovirus target	Cy5 channel RNA IPC target	Interpretation
Negative control	Negative	Negative / Positive <sup>1</sup>	Valid
Positive control	Positive	Negative / Positive <sup>1</sup>	Valid
Extraction negative control	Negative	Positive	Valid
Sample	Positive	Positive / Negative <sup>2</sup>	Positive
Sample	Negative	Positive	Negative <sup>3</sup>
Sample	Negative	Negative	Invalid

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

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

<sup>3</sup>The 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 channel 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 channel 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 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.

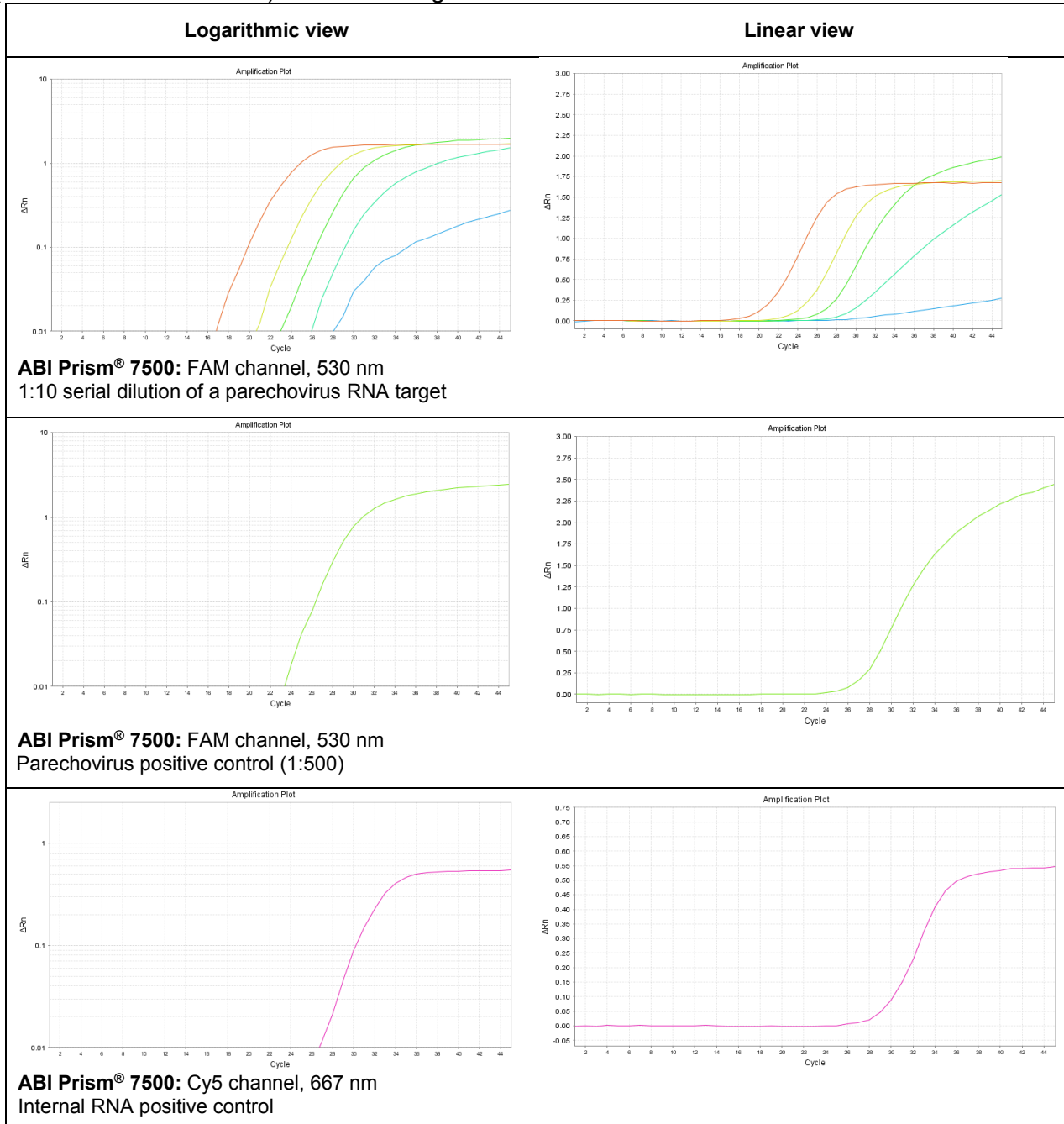
### 11.4. Signal in FAM channel in extraction negative control

- 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 Parechovirus with an Applied Biosystems® 7500 Fast Real-time PCR System (Thermo Fisher Scientific) is shown in Figure 1.



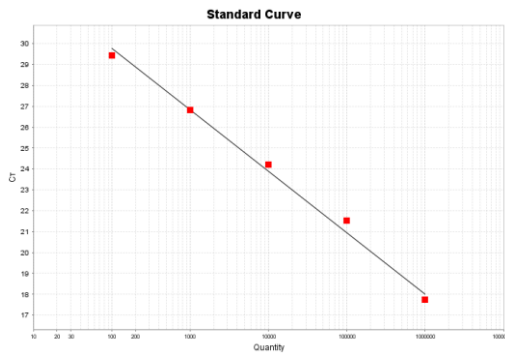
**Figure 1** Performance of ViroReal® Kit Parechovirus



## 12.2. Analytical sensitivity and linearity

The analytical sensitivity is about 100 RNA copies/PCR.

The assay shows **linearity** over the range of 100 to 1,000,000 target copies/reaction as shown in Figure 2.



**Figure 2** Ten-fold dilution series of parechovirus RNA plotted against CT

## 12.3. 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 parechovirus strains.

## 13. References

Harvala H, Simmonds P. 2009. Human parechoviruses: biology, epidemiology and clinical significance. *J Clin Virol.* 45:1-9.