ViroReal® Kit Parainfluenzavirus

Kit version 1.1

For *in vitro* diagnostic use only

ViroReal® Kit Parainfluenzavirus			
Order no.	**Reactions**	**Pathogen**	**Internal positive control**
DHUV01653	50	FAM + VIC channel	Cy5 channel

Kit contents:

- Assay for detection of parainfluenzavirus types 1, 2, 3, 4 and of internal RNA positive control (IPC)
- IPC-Target RNA (control of RNA extraction and of RT PCR amplification)
- RNA reaction mix for one-step reverse transcription real-time PCR (contains a thermostable MMLV Reverse Transcriptase, a 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)
- RNA positive control for parainfluenzavirus 1 and 2
- Nuclease-free water

Pathogen information: Human parainfluenzaviruses (HPIV) are enveloped, negative single-stranded RNA viruses (ss(-)RNA) of the family *Paramyxoviridae*. At present four serotypes are known (types 1, 2, 3, 4). Parainfluenzavirus type 1 and 3 belong to the genus *Respirovirus*, while parainfluenzavirus type 2 and 4 belong to the genus *Rubulavirus.*
Human parainfluenzavirus type 1 (HPIV-1) is the major cause of acute croup but can also cause respiratory tract infections at all ages. Human parainfluenzavirus type 2 (HPIV-2) can also cause croup as well as upper and lower respiratory tract illnesses. Human parainfluenzavirus type 3 (HPIV-3) causes bronchiolitis and pneumonia. Human parainfluenzavirus type 4 with the subtypes 4a and 4b is less likely to cause servere disease.

Intended purpose: ViroReal® Kit Parainfluenzavirus is a non-automated CE-certified IVD test, based on one-step reverse transcription real-time PCR (RT-PCR), for the qualitative detection of RNA of the HN gene of parainfluenzavirus 1, 2, 3 and 4 strains (HPIV-1, HPIV-2, HPIV-3, HPIV-4).
Proper specimens are RNA extracts isolated from samples of the human respiratory tract.
This test is suitable for patients of all ages with or without a suspected parainfluenzavirus 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.
A probe-specific amplification-curve in the FAM channel indicates the amplification of parainfluenzavirus type 1 and 3 (HPIV-1 and HPIV-3) specific RNA, while a probe-specific amplification-curve in the VIC channel indicates the amplification of parainfluenzavirus type 2 and 4 (HPIV-2 and HPIV-4) specific RNA. An 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.

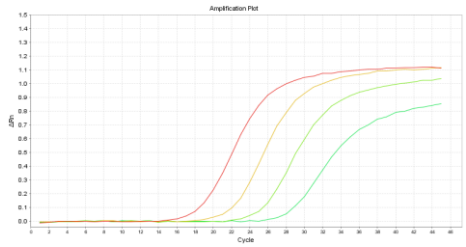
PCR-platforms: This test has been validated with the ABI® 7500 Fast Real-time PCR System (Thermo Fisher Scientific) and tested with a LightCycler® 480 Instrument II (Roche), Mx3005P® QPCR System (Agilent), MIC instrument (bio molecular systems), QuantStudio™ 7 Pro (Thermo Fisher Scientific) and qTOWER^{3}G (Analytik Jena).

It is also compatible with other real-time PCR instruments which detect and differentiate fluorescence in FAM, VIC and Cy5 channel (e.g., QuantStudio™ 5 (Thermo Fisher Scientific), cobas z 480 Analyzer (Roche)).

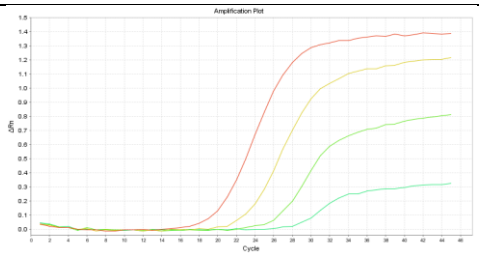
Performance data: ViroReal® Kit Parainfluenzavirus detects at least 10 target copies/reaction of HPIV-1 and at least 100 target copies/reaction of HPIV-2, HPIV-3, and HPIV-4. The LoD95% (smallest number of copies of target RNA which can be detected in 95% of cases) is 47 target copies/reaction for HPIV-1, 335 target copies/reaction for HPIV-2, 294 target copies/reaction for HPIV-3 and 346 target copies/reaction for HPIV-4.
Diagnostic evaluation was performed with different viral isolates, with various parainfluenzavirus 1, 2, 3 and 4 isolates and with clinical samples. This kit is specific for so far known HPIV-1, HPIV-2, HPIV-3, HPIV-4 strains.

Results of clinical validation:

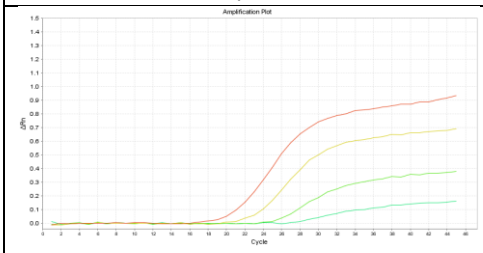
	Value	95% CI
Sensitivity	100%	87.23% to 100%
Specificity	100%	78.20% to 100%
NPV	100%	78.20% to 100%
PPV	100%	87.23% to 100%
Prevalence	64%	



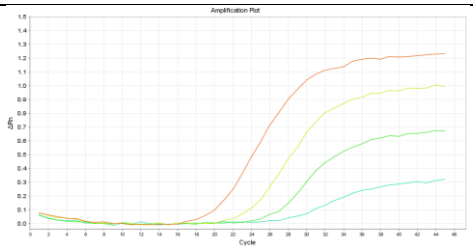
FAM channel:
1:10 serial dilution of a parainfluenzavirus 1 RNA target



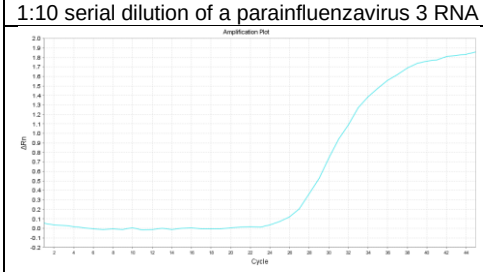
VIC channel:
1:10 serial dilution of a parainfluenzavirus 2 RNA target



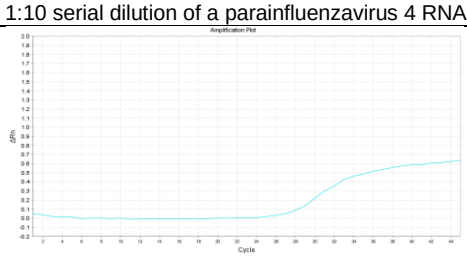
FAM channel:
1:10 serial dilution of a parainfluenzavirus 3 RNA target



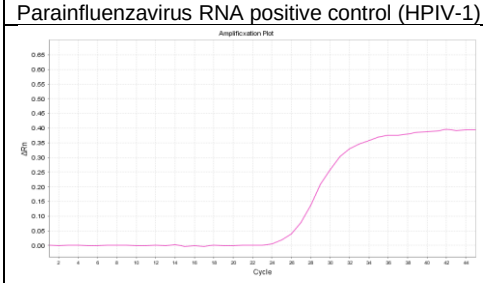
VIC channel:
1:10 serial dilution of a parainfluenzavirus 4 RNA target



FAM channel:
Parainfluenzavirus RNA positive control (HPIV-1)



VIC channel:
Parainfluenzavirus RNA positive control (HPIV-2)



Cy5 channel: Internal RNA positive control

Figure 1 Performance of ViroReal® Kit Parainfluenzavirus