ViroReal® Kit Influenza A/B

Kit version 1.1

For *in vitro* diagnostic use only

ViroReal® Kit Influenza A/B			
Order no.	**Reactions**	**Pathogen**	**Internal positive control**
DHUV00253	50	FAM+VIC channel	Cy5 channel

Kit contents:

- Assay for detection of influenza virus A (FAM) + influenza virus B (VIC) 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 influenza virus A and B
- Nuclease-free water

Pathogen information: Influenza is an acute infectious disease caused by influenza virus A, B or, to a much lesser extent, influenza virus C. Influenza viruses are enveloped viruses with single-stranded, segmented RNA with negative polarity as genome. These viruses can be found worldwide. Epidemics and pandemics are mainly caused by influenza virus A, due to antigenic drift of the hemagglutinin and neuraminidase molecules. Type B and C influenza viruses are isolated almost exclusively from humans, while influenza A viruses infect a wide variety of warm-blooded animals.

Intended purpose: ViroReal® Kit Influenza A/B 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 matrix protein gene of influenza A virus and the hemagglutinin gene of influenza B virus.
Proper specimens are RNA extracts of samples from the human upper respiratory tract (nasal swabs, nasopharyngeal swabs, and oropharyngeal swabs).
This test is suitable for patients of all ages with suspected influenza A or B virus infection and is intended as an aid in the diagnosis of these pathogens 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.
Influenza virus A RNA is detected in FAM channel, influenza virus B RNA in VIC channel. The internal RNA positive control (RNA IPC) is detected in Cy5 channel and is used as RNA extraction as well as RT-PCR inhibition control. The RNA IPC target is extracted with the sample.

PCR-platforms: This test has been validated with the ABI® 7500 Fast instrument (fast cycle parameters are not supported, Thermo Fisher Scientific) and was also tested with QuantStudio™ 7 Pro (Thermo Fisher Scientific), MIC instrument (bio molecular systems) and LightCycler® 480 II (Roche Diagnostics). 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, Mx3005P® (Agilent), qTOWER^{3}G (Analytik Jena), cobas z 480 Analyzer (Roche)).

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Performance data: The LoD95% (smallest number of copies of target RNA which can be detected in 95% of cases) is 5.7 target copies/reaction for influenza A virus and 6.0 target copies/reaction for influenza B virus. This kit is specific for so far known influenza A and B strains.
For diagnostic evaluation, 24 influenza A isolates and 6 nasopharyngeal swabs positive for influenza A were tested with an Assay Mix containing primers and probe for the detection of the matrix protein gene of influenza A virus (singleplex detection). Furthermore, 9 influenza B isolates, 4 nasopharyngeal swabs positive for influenza B and 13 nasopharyngeal swabs negative for influenza B were tested with an Assay Mix containing primers and probe for the detection of the hemagglutinin gene of influenza B virus (singleplex detection). See Table 1.

Table 1 Summary diagnostic evaluation

	Influenza virus A		Influenza virus B	
	Value	**95% CI**	**Value**	**95% CI**
Sensitivity	100%	88.43% to 100%	100%	75.29% to 100.00%
Specificity	100%	85.18% to 100%	100%	88.43% to 100.00%
NPV	100%	85.18% to 100%	100%	88.43% to 100.00%
PPV	100%	88.43% to 100%	100%	75.29% to 100.00%
Prevalence	64%		30%	

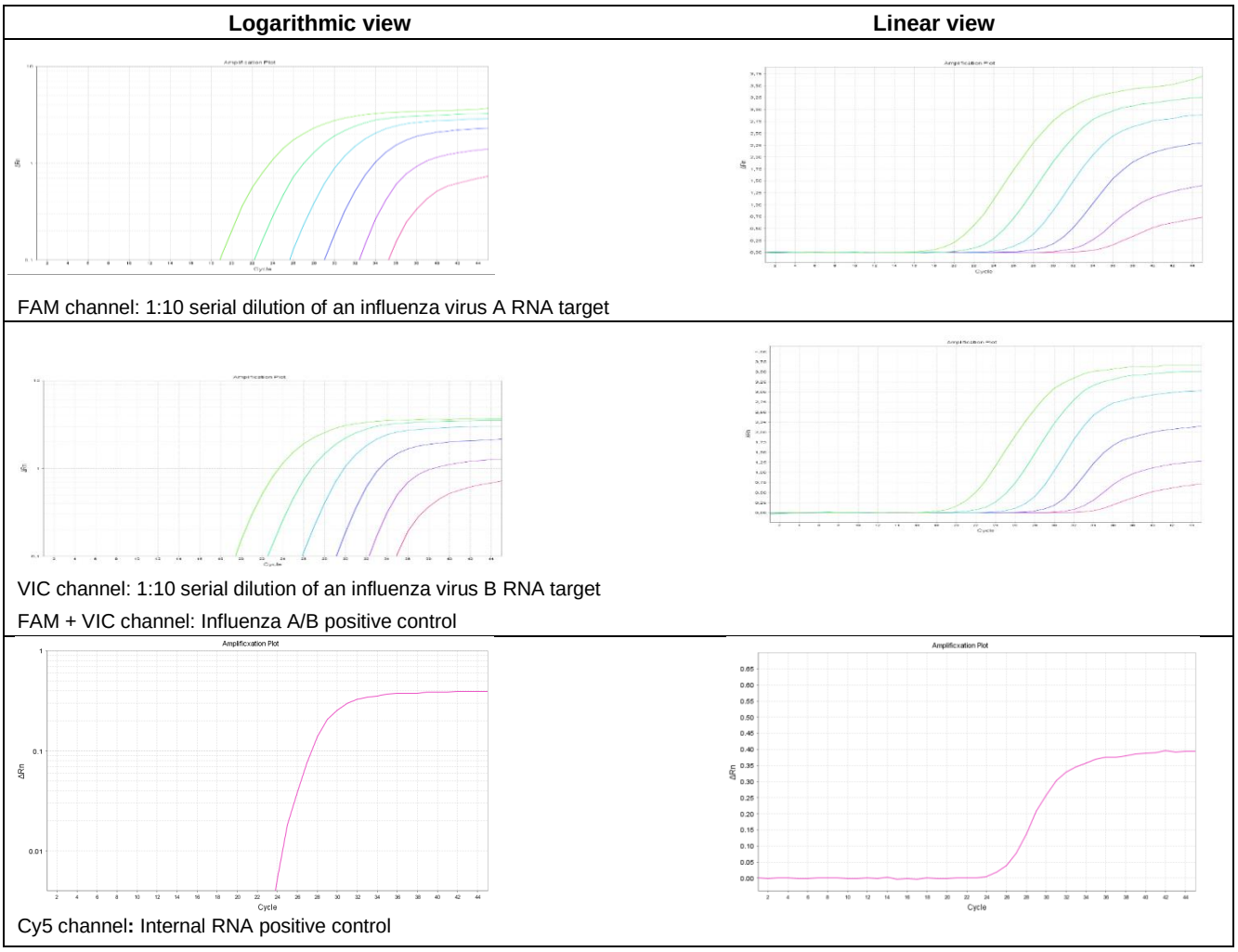

Figure 1 Performance of ViroReal® Kit Influenza A/B