ViroReal® Kit Enterovirus

Kit version 1.1

For *in vitro* diagnostic use only

ViroReal® Kit Enterovirus			
Order no.	**Reactions**	**Pathogen**	**Internal positive control**
DHUV00353	50	FAM channel	Cy5 channel

Kit contents:

- Assay for detection of enterovirus 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 enterovirus
- Nuclease-free water

Pathogen information: Enteroviruses are non-enveloped, positive single-stranded RNA viruses (ss(+)RNA) of the family *Picornaviridae*. The genus *Enterovirus* has a worldwide distribution and includes numerous viral groups infecting humans, bovines, equines, simians, porcines and rodents.
Enteroviruses infecting humans are poliovirus, coxsackievirus, echovirus, human enteroviruses, and human rhinoviruses. Enteroviruses are the causative agents e.g., for infection of the respiratory tract (summer flu, pharyngitis, pneumonia) and infection of inner organs (myocarditis, encephalitis, pleurodynia), aseptic meningitis, herpangina, hand-foot-mouth disease, haemorrhagic conjunctivitis, and poliomyelitis.

Intended purpose: ViroReal® Kit Enterovirus 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 (5´UTR region) of the genus *Enterovirus*. The detection of following serotypes has been confirmed: Coxsackie-Virus A1, coxsackie-virus A5, coxsackie-virus A6, coxsackie-virus A13, coxsackie-virus A21, coxsackie-virus B2, coxsackie-virus B3, coxsackie-virus B5, echovirus 1, echovirus 6, echovirus 11, echovirus 14, echovirus 30, enterovirus 71, enterovirus 90, enterovirus 99, enterovirus D68, poliovirus type 1, poliovirus type 2 and poliovirus type 3. Some rhinoviruses can also be detected, but with expected lower sensitivity.
Proper specimens are RNA extracts isolated from samples of the human respiratory tract (nasopharyngeal swabs) or stool. This test is suitable for patients of all ages with suspected enterovirus infection and is intended as an aid in the diagnosis of enterovirus infection 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 enterovirus 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.

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

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), Mx3005P® (Agilent), qTOWER^{3}G (Analytik Jena), 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 and Cy5 channel (e.g., QuantStudio™ 5, cobas z 480 Analyzer (Roche)).

Performance data: ViroReal® Kit Enterovirus detects at least 10 target copies/reaction. The LoD95% (smallest number of copies of target RNA which can be detected in 95% of cases) is 30.6 target copies/reaction. This kit is specific for so far known enterovirus strains. Evaluation was performed with viral isolates and clinical samples (nasopharyngeal swabs and stool samples). The detection of following enterovirus serotypes has been confirmed: Coxsackie-Virus A1, coxsackie-virus A5, coxsackie-virus A6, coxsackie-virus A13, coxsackie-virus A21, coxsackie-virus B2, coxsackie-virus B3, coxsackie-virus B5, echovirus 1, echovirus 6, echovirus 11, echovirus 14, echovirus 30, enterovirus 71, enterovirus 90, enterovirus 99, enterovirus D68, poliovirus type 1, poliovirus type 2 and poliovirus type 3. Some rhinoviruses are detected as well, but with expected lower sensitivity.

Results of clinical validation:

	Value	95% CI
Sensitivity	100%	92.89% to 100.00%
Specificity	100%	90.75% to 100.00%
NPV	100%	90.75% to 100.00%
PPV	100%	92.89% to 100.00%
Prevalence	57%	

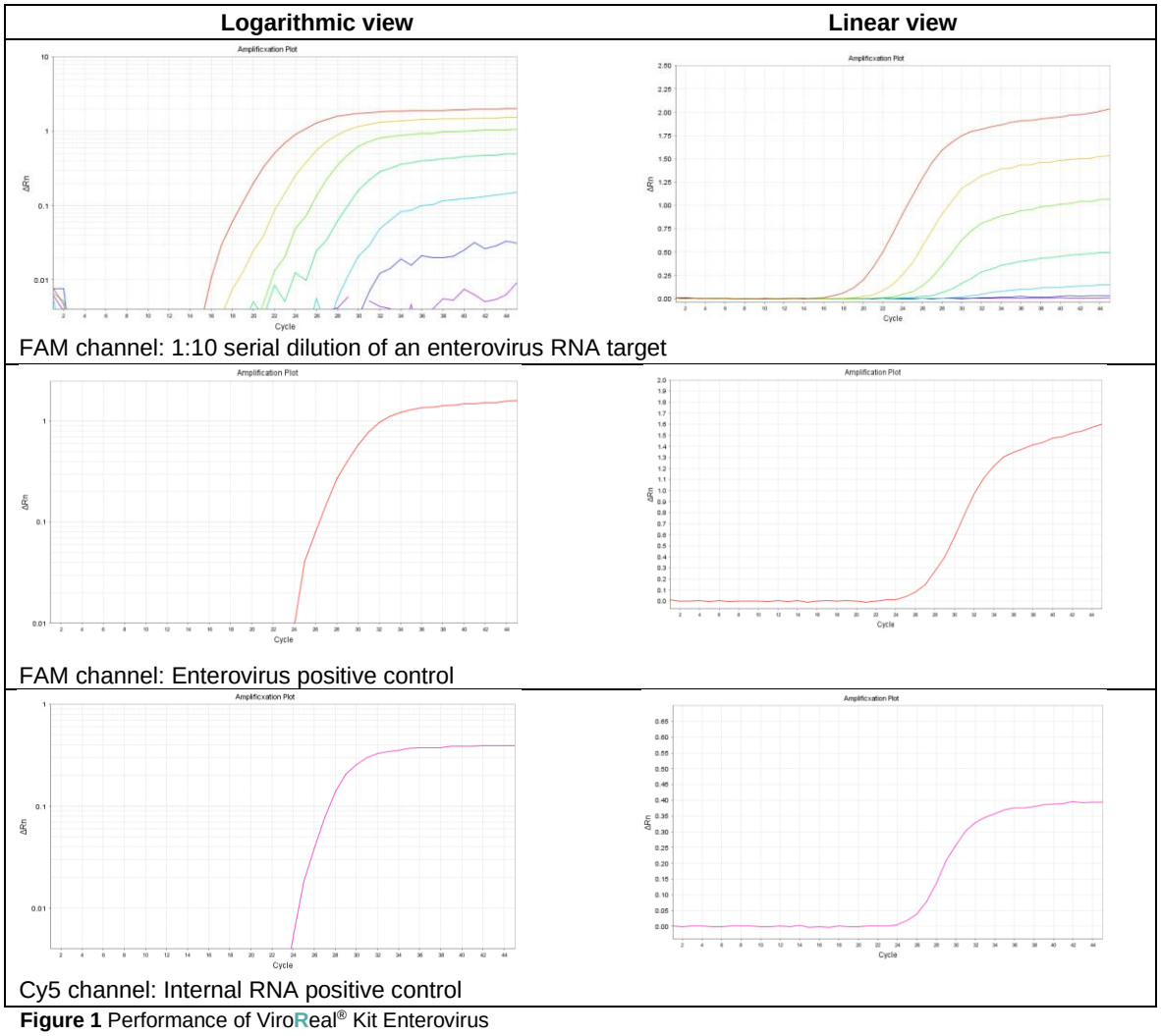

Figure 1 Performance of ViroReal® Kit Enterovirus