ViroReal® Kit SARS-CoV-2 Multiplex

Kit version 2.1

For *in vitro* diagnostic use only

ViroReal® Kit SARS-CoV-2 Multiplex			
Order no.	**Reactions**	**Pathogen**	**Internal positive control**
DHUV02413	100	FAM+VIC channel	Cy5 channel
DHUV02413x5	500	FAM+VIC channel	Cy5 channel

Kit contents:

- Assay for detection of N gene (FAM) + RdRp gene (VIC) of SARS-CoV-2 and of internal RNA positive control (IPC) (Cy5)
- 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 SARS-CoV-2
- Nuclease-free water

Pathogen information: Coronaviruses are positive single-stranded 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, SARS-CoV-2, 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, a beta coronavirus, causes the Severe Acute Respiratory Syndrome (SARS).
SARS-CoV-2, a beta corona virus, causes COVID-19 (corona virus disease 2019). Fever, cough, and breathing difficulties are described as the most common initial symptoms, and infection can subsequently lead to pneumonia. The coronavirus spreads mainly by droplet and contact transmission.

Intended purpose: ViroReal® Kit SARS-CoV-2 Multiplex 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 N gene of SARS-CoV-2, SARS-CoV and SARS-related coronavirus (universal detection of Sarbecovirus) and of the RdRp gene of SARS-CoV-2 (specific detection of SARS-CoV-2).
Proper specimens are RNA extracts isolated from samples of the human respiratory tract (throat rinsing fluid, nasopharyngeal and oropharyngeal swabs, anterior nasal swab and mid-turbinate nasal swab specimens, nasopharyngeal wash/aspirate, and nasal aspirates).
This test is suitable for patients of all ages with or without a suspected SARS-CoV-2 or SARS coronavirus 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.
RNA of the N gene is detected in FAM channel, RNA of the RdRp gene 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), Mx3005P® (Agilent), qTOWER³G (Analytik Jena) 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 (Thermo Fisher Scientific), cobas z 480 Analyzer (Roche)).

Performance data: The LoD95% (smallest number of copies of target RNA which can be detected in 95% of cases) is 6 target copies/reaction for the N gene and 5 target copies/reaction for the RdRp gene.
For the N gene, a highly conserved region in all SARS coronavirus clusters (SARS-CoV-2 as well as SARS-CoV and SARS-related bat coronavirus) was chosen as target region. For the RdRp gene, a highly specific region for SARS-CoV-2 was chosen, the RdRp gene of other coronaviruses are not detected, with exception of Bat coronavirus RaTG13, which is the coronavirus most closely related to SARS-CoV-2. For clinical valuidation, extracts from 275 swab samples from the upper respiratory tract or gargeling samples were examined.

Results of clinical validation:

	Value
Sensitivity	100 %
Specificity	100 %
NPV	100 %
PPV	100 %

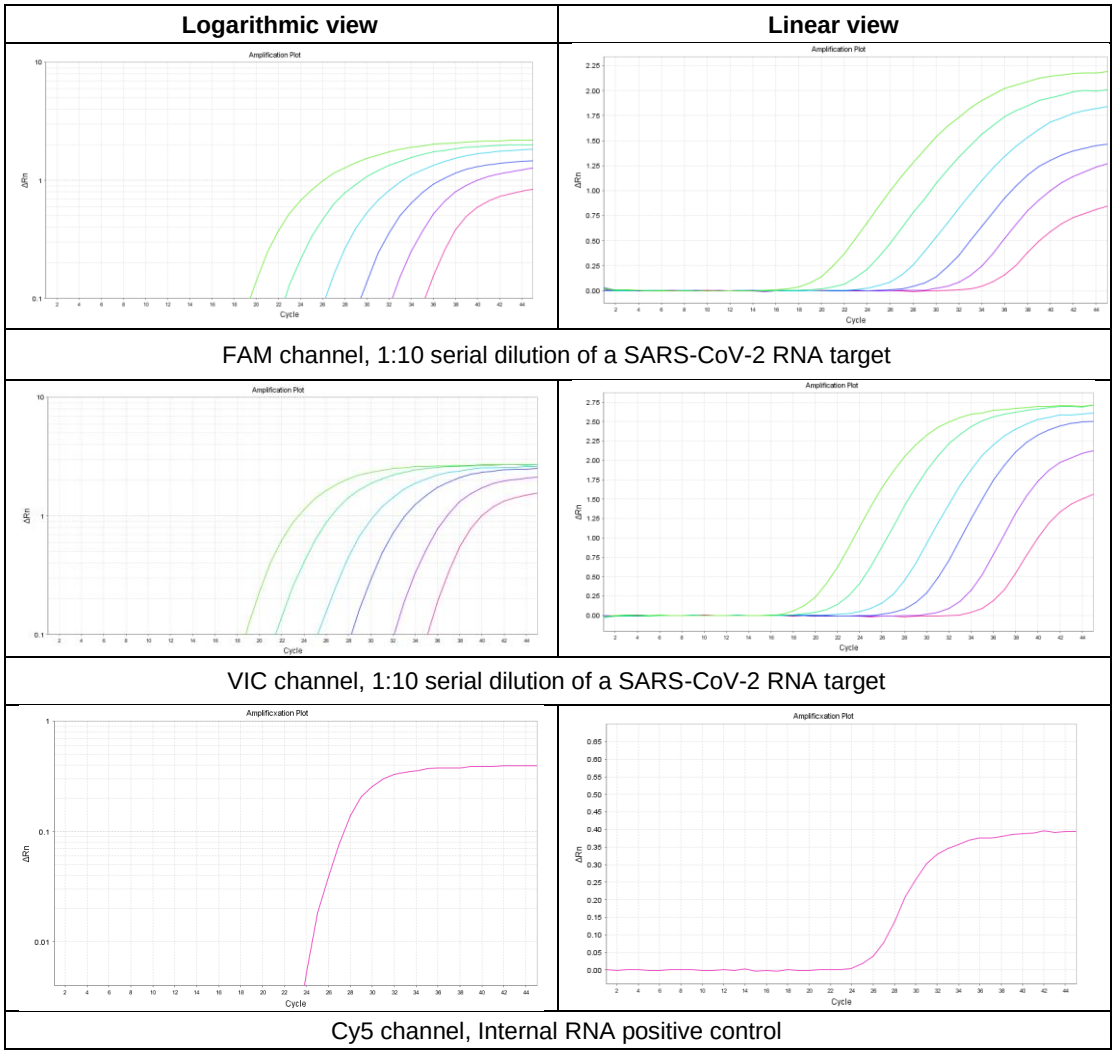

Figure 1 Performance of ViroReal® Kit SARS-CoV-2 Multiplex