DIACARTA

QUICK START GUIDE

QuantiVirus™ SARS-CoV-2 Multiplex Detection Test

Step 1. Viral RNA Isolation

For each batch of clinical samples to be tested, it is necessary to have one extraction control. Spike 2 uL of Extraction control (EC) from the QuantiVirus™ kit into 198 uL sterile RNase-free water prior to RNA extraction. Extract RNA from spiked Extraction control and clinical samples following the extraction kit manufacturer's instructions. Check the concentrations of viral RNA Extractions and make sure the A260/A280 values are around 2.0.

Step 2. Preparation of Reagents and Assay Mixes

- Thaw all reaction mixes at room temperature for a minimum of 30 minutes
- Gently invert the vials of qRT-PCR Master Mix and Primer/Probe Mixes a few times
- Vortex all other kit components for 5 seconds and perform a quick spin
- Pre-mix the qRT-PCR Master Mix and Primer/Probe Mixes into Assay Mixes with proper volumes according to the number of samples for qRT-PCR detection. Follow the guideline as shown in the table below:

Reagents	Assay Mix
qRT-PCR Master Mix	2.5 µl x (n+3+1)
Primer/Probe Mix	2.0 µl x (n+3+1)
Total Volume	4.5 µl x (n+3+1)

- Number of reactions of Mix = n (Viral RNA samples) + 3(PC/NTC/EC) +1
- Prepare enough volume for 1 extra reaction to compensate for pipetting loss in qRT-PCR setup.

Step 3. Sample and Control Input

For viral RNA Sample and Control template input of each 10 uL reaction mix, follow the guideline as shown in the table below:

Components per Reaction	Viral RNA Sample	PC/NTC/EC Control Reaction
Assy Mixes	4.5 µl	4.5 µl
RNA samples or Controls	5.5 µl *	2.0 µl
Nuclease-Free Water	0.0 µl **	3.5 µl
Total volume	10.0 µl	10.0 µl

*Maximum viral RNA Sample input is 5.5 µl per reaction.
**Bring total volume of each reaction to 10.0 µl when Viral RNA Sample input is less than 5.5 µl per reaction.

Step 4. Instrument Set-Up

After loading all Assay Mixes into qRT-PCR plate, tightly seal the plate to prevent evaporation. Spin plate at 1000 rpm for 1 minute to collect all reagents and place in the real-time PCR instrument immediately. Set up the thermocycling program on the qPCR instrument according to tables below.

ABI QuantStudio 5 and ABI 7500 Fast Dx (ORF1ab in FAM channel; Rp in HEX/VIC channel)

Stages	Temperature (°C)	Time (Seconds)	Ramp Rate (°C/s)	Cycles	Data Collection
UNG incubation	25	120	1.6	1	OFF
Reverse Transcription	53	600	1.6	1	OFF
Polymerase activation	95	120	1.6	1	OFF
Denaturation	95	3	1	X45	OFF
Annealing and Extension	60	30	1		FAM, HEX

**** For ABI 7500 FAST Dx, please use FAST mode with automatic ramp rate.**

Step 5. Data Analysis

Refer to “Product Instruction for Use (IFU)”