

Step 1. Viral RNA Isolation

For each batch of clinical samples to be tested, it is necessary to have one extraction control. Spike 20 µL of Extraction control (EC) from the QuantiVirus™ kit into 180 µL 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 extracted viral RNA 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
- Keep all thawed reagents and samples on ice while setting up the reaction mixes
- 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 µL reaction mix, follow the guideline as shown in the table below:

Components per Reaction	Viral RNA/EC Sample Reaction	PC/NTC 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 programs on the qPCR instruments 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.

Bio-Rad CFX 384 (Select all channels)

RT-qPCR thermocycling parameters for Bio-Rad CFX 384	Temperature (°C)	Time (Seconds)	Cycles	Data Collection
UNG incubation	25	120	1	OFF
Reverse Transcription	53	600	1	OFF
Polymerase activation	95	120	1	OFF
Denaturation	95	3	45	OFF
Annealing and Extension	60	30		FAM, HEX

Step 5. Data Analysis

Refer to “Product Instruction for Use (IFU)”