ViPrimePLUS 2019-nCoV Multiplex RT-qPCR Kit

Quantitative assay for real-time RT-PCR detection of
2019-nCoV

Product Code: QM50811
Pack Size: 100 reactions

vivantis

INTENDED USE
The ViPrimePLUS 2019-nCoV RT-qPCR Kit is a Taqman probe-based real-time PCR assay for the detection of 2019-nCoV genome in clinical samples (e.g. nasopharyngeal and oropharyngeal swab, bronchoalveolar lavage, tracheal aspirate, nasopharyngeal aspirate or nasal wash, sputum and serum.). This assay is intended for research use only.

INTRODUCTION
2019-nCoV is a novel strain of Coronavirus that caused an outbreak of pneumonia in Wuhan City (China) on the end of December 2019. This virus belongs to the same family of Severe Acute Respiratory Syndrome (SARS) and Middle Eastern Respiratory Syndrome (MERS) viruses. This positive-sense, single-stranded RNA virus is thought to be of zoonotic origin and can be transmitted from human to human. Individuals infected with this virus would show symptoms like fever, dry cough, fatigue, pneumonia, shortness of breath and respiratory distress. Recently, there were cases reported to have cause renal failure, pneumonia and death. In additions, patients with impaired immune systems are the most seriously affected by 2019-nCoV; even some of the healthy individuals have required intensive care once infected.

PRINCIPLE OF TEST
The kit contains primers and Taqman® probe that target the 2019-nCoV genomes. In this one-step real-time RT-PCR, reverse transcription of this viral RNA is combined with the qPCR step in a single tube reaction. This closed-tube assay reduces the chances of contamination and improves the sensitivity of the test.

Based on the Taqman® probe detection principle, the 5'-reporter dye and 3'-quencher dual-labelled oligonucleotide (Taqman® probe) hybridizes on a specific region within the amplified fragment. During amplification, the probe is cleaved and the reporter dye (fluorophore) is released. The fluorescent signal intensity detected is proportional to the number of amplicons. The Ct value (the cycle at which the rise of fluorescent signal from the baseline is first significant) is used for quantification purposes. Targets pathogen amplification are detected using FAM, Texas Red and Cy5 channels.

The kit provides the Internal Control (IC) as inhibition control. An IC specific primers and probe labelled with a different dye is provided to be run in the same reaction with the pathogen-specific primers and probe mix. The IC amplicon is detected via HEX channel at Ct value 28±3 depending on the sample dilution.

A positive control with known copy number is provided for standard curve construction and absolute quantification. It can also be used at a single dilution for qualitative analysis control of the experimental set-up. Extra care must be taken to avoid cross-contamination.

QUALITY CONTROL
Each lot of ViPrimePLUS 2019-nCoV RT-PCR Kit has been tested against predetermined specifications to ensure consistent product quality under ISO 9001:2015 – certified Quality Management System.

SENSITIVITY & SPECIFICITY
The detection limit is tested to 100 copies per reaction. The primers and probe are 100% specific.

STORAGE & STABILITY
Store at -20^{o}C and avoid light exposure. Stable at -20°C up to the expiry date stated. Keep in aliquot to reduce freeze-thaw cycles.

LIMITATION OF TEST
For research use and diagnostic use only.
Result is dependent on the yield and quality of the nucleic acids extracted from the method of extraction. Thus, it is important to do spectrophotometric and gel analysis on the extracted samples.

KIT COMPONENTS

Ready-to-go One Step RT-qPCR Pre-mixed	Amber Tube	1.0ml
Positive Control	White Capped Tube	0.1ml
Nuclease Free Water	White Capped Tube	1.0ml
ROX Dye (Low ROX)	Amber Tube	0.5ml
ROX Dye (High ROX)	Amber Tube	0.5ml

SAMPLE MATERIAL
The kit is suitable for RNA extracted by most commercial kits, provided the purity, concentration and integrity are within acceptable range. Suitable sample types are nasopharyngeal and oropharyngeal swab, bronchoalveolar lavage, tracheal aspirate, nasopharyngeal aspirate or nasal wash, sputum and serum.

Reconstitution of Ready-to-go One Step RT-qPCR Pre-mixed
Add 500µl PCR Water into Pre-mixed tube. Mix well and short spin.

Reconstitution of Ready-to-go One Step RT-qPCR Pre-mixed (with ROX)
Add 500µl provided ROX dye into Pre-mixed tube. Mix well and short spin.

PROTOCOLS

Real-time PCR reaction set-up

Recommended real-time PCR reaction set-up:

Reagents	1 reaction (µl)
Ready-to-go One Step RT-qPCR Pre-mixed	15
Sample RNA/Positive control	5
TOTAL	**20**

**Suggested sample concentration 5-20ng/µl.*
**Adjust nuclease-free water to make up the final reaction volume of 20µl.*
**Prepare an extra reaction to accommodate for pipeting error*
**Set aside one tube/well as negative control where 5µl of nuclease-free water is used as the template. This is also known as no template control.*

Set the thermal cycler parameters as follows:

Step	Time	Temp	Cycles	Scan
Reverse transcription	10mins	42°C		
Enzyme activation	2mins	95°C		
Denaturation	15secs	95°C	50	
Anneal/Elongation	1min	60°C		√*

***FAM, HEX, Cy5 & Texas Red**

INTERPRETATION OF RESULTS

Pathogen specific amplification signal is detected via FAM, Texas Red & Cy5 channel, while IC amplification is detected via HEX channel.

The cut off is set at CT value 40. The signal is positive if the amplification curve crosses the threshold line. The result is relevant provided both positive and negative controls give valid results.

Summary of interpretation:

Target 1 (FAM/N)	Target 2 (Texas Red/RdRP)	Target 3 (Cy5/E)	IC	Negative Control	Positive Control	Interpretation
+	+	+	+/-	-	+	Valid. 2019-nCoV detected
If only 1 target is positive			+/-	-	+	Presumptive Positive. Repeat testing of RT-qPCR and/or re-extract sample and repeat RT-qPCR and/or consider collecting a new specimen from the patient.
-	-	-	+	-	+	Valid. 2019-nCoV not detected
-	-	-	-	-	-	Invalid. Repeat extraction and RT-qPCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.

*Positive control template is expected to amplify between Ct 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

Internal Control

When used accordingly and assuming 100% extraction efficiency, a Ct value of 28±3 is within normal range. High 2019-nCoV genome copy amplification may out compete the IC amplification. Thus, the latter may not produce an amplification signal. The positive result is still valid in this case.

TROUBLESHOOTING

Problem	Possibility	Suggestion
Negative control / No template control gives positive result	Carry over contamination	Change nuclease-free water. Use fresh aliquots of reagents. Use filtered tips. Load positive control last.
No signal detected from positive control	Incorrect programming of instrument	Check program.
	Reagents expired	Check the expiry date of reagents before repeat.
	Storage condition not complying with instructions	Check storage condition properly and store at correct storage condition to avoid the degradation of reagents.
	Pipetting error	Pipette the correct volume of reagents to reconstitute the components of kit and mix well.
Internal extraction control does not give a signal in apparently negative samples	Inhibitors in the samples extracted	Repeat the extraction.
	Low recovery of RNA extracted	Repeat the extraction by enlarge the sample size.
	IEC added directly into unprocessed biological sample – lead to degradation and loss of signal	Add IEC into each sample suspended in the lysis/extraction buffer.

DEVIATION OF MASTERMIX FORMULATION

Manufacturers use varying methods to calibrate a real-time PCR reaction. For this reason, we provide several Mastermix formulations for those platforms.

Master Mix	Compatible Hardware
Original	Analytik Jena qTower series, biorad iCycler all series, BioRad CFX96 & CFX384, Cepheid SmartCycler®, Eppendorf Mastercycler series, Fluidigm BioMark™, Illumina Eco, MJ Chromo4, Opticon, PCRMax Eco™, Roche lightcycler® series, Qiagen RotorGene, Thermo PikoReal™
Low Rox (-LR)	Agilent / Stratagene Mx3000, Mx3005P, Mx4000, Mx4000R, Applied Biosystems 7500 and 7500 FAST platform, QuantStudio™, ViiA7.
Rox (-R)	Applied Biosystems 7000, 7300, 7700, 7900 and 7900HT FAST platforms, OpenArray, PRISM 7000, 7700, 7900, GeneAmp® 5700, StepOne™, StepOne™ PLUS

WARRANTY & LIMITED LIABILITY

The performance characteristics stated were obtained using the assay procedure in this insert. Failure to comply with the instructions may derive inaccurate results. In such event, manufacturer disclaims all warranty expressed, implied or statutory including the implied warranty of merchantability and the fitness of use.

The manufacturer will not be liable for any damage caused by misuse, improper handling and storage; non-compliance with precautions and procedures, and damages caused by events occurring after the product is released.