PowerChek™ 2019-nCoV Real-time PCR Kit

Cat No. R6900T (50 samples)

Description

PowerChek™ 2019-nCoV Real-time PCR Kit provides the fast and accurate testing solution for Wuhan coronavirus, specifically targeting the E gene for beta Coronavirus and the RdRp gene for 2019-nCoV in bronchoalveolar lavage fluid, sputum, nasopharyngeal swab and oropharyngeal swab.

Our 2019-nCoV Real-time PCR assay is based on the WHO & KCDC reference method and it has been carried out the *in-sillico* analysis for all registered 2019-nCoV sequence database.

The kit is a specific ready-to-use detection for beta coronavirus and 2019-nCoV by one-step Real-time RT-PCR system. We offer the complete solution kit including specific primer and probe, one-step RT-PCR premix, positive amplification control and internal control as well.

Kit components

(Store at -20℃)

Component parts	Cap	Volume (㎕)
RT-PCR Premix	RP	1,100 ㎕
Primer/Probe Mix 1 (E gene)	P1	200 ㎕
Primer/Probe Mix 2 (RdRp gene)	P2	200 ㎕
Control 1 (E gene)	C1	50 ㎕
Control 2 (RdRp gene)	C2	50 ㎕

Protocol

※ We recommend that all experiment steps be performed wear the poly-glove to prevent the risk of contamination with RNase.

1. **RNA Isolation**. Various manufacturer offer RNA isolation kits. Carry the RNA isolation according to the manufacturer's instructions.
   ※ Recommend : E0007 PowerPrep™ Viral DNA/RNA Extraction Kit

2. **Thawing the kit components on ice .** Using ice or lap top cooler is recommended during experiment for maintaining the enzyme activity. Spin-down the tubes before use.

3. **Preparing PCR Mixture.** Total reaction volume is 20 ㎕, the volume of RNA sample is 5 ㎕. **Prepare a reaction mixture according to the table below.**

※ Positive control : Add 5 ㎕ of Control instead of sample RNA.
※ Negative control : Add 5 ㎕ of Nuclease free water instead of sample RNA.

Component	Volume (㎕)
RT-PCR Premix	11
Each Primer/Probe Mix	4
Template RNA	X (control 5 ㎕)
Total	20

4. Mix the reagents in the PCR reaction tubes by tapping minimum of 5 times. Briefly centrifuge the tubes to remove air bubble and drops from the inside of the cap.

5. **Real-time PCR run.** Program the instruments according to manufacturer's manual. Create a temperature profile on your instrument as follows the table below.

Temperature (℃)	Time	Cycles
50 ℃	30 min	1
95 ℃	10 min	1
95 ℃	15 sec	40
60 ℃	1 min	

Data Analysis

- The fluorescence curves are analyzed on FAM fluorescence detection channel in the table below.

Fluorophore	Target
FAM	E gene – beta coronavirus
JOE (VIC or HEX)	IC (Internal Control)

Fluorophore	Target
FAM	RdRp gene – 2019-nCoV
JOE (VIC or HEX)	IC (Internal Control)

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Interpretation

- The signal is considered to be positive, if the corresponding fluorescence accumulation curve cross threshold line. Results are accepted as relevant if both positive and negative controls of amplification are passed.

<E gene assay>

Case	Positive Control	Negative Control	FAM	JOE	Interpretation
			E gene	IC	
1	+	-	+	+	beta coronavirus detected
2	+	-	+	-	
3	+	-	-	+	beta coronavirus not detected
4	+	-	-	-	Invalid result / Retest
5	+	+	+/-	+/-	
6	-	+	+/-	+/-	
7	-	-	+/-	+/-	

<RdRp gene assay>

Case	Positive Control	Negative Control	FAM	JOE	Interpretation
			RdRp gene	IC	
1	+	-	+	+	2019-nCoV detected
2	+	-	+	-	
3	+	-	-	+	2019-nCoV not detected
4	+	-	-	-	Invalid result / Retest
5	+	+	+/-	+/-	
6	-	+	+/-	+/-	
7	-	-	+/-	+/-	

- Detection of the internal amplification control in the JOE detection channel is not required for positive result.
- High copy number of target gene can lead to reduced or absent internal amplification control signal.