GeneFinder™ COVID-19 Plus RealAmp Kit

REF **IFMR-45** **Σ 100 tests/Kit**

Store at -20℃ or below.
Shelf life is 12 months after manufacturing.

IVD
For ABI7500/7500Fast & CFX96

INTENTED USE

GeneFinder™ COVID-19 Plus RealAmp Kit is the One-Step Reverse Transcription Real-Time PCR Kit designed to detect Novel Corona virus (COVID-19) qualitatively through Reverse Transcription reaction and Real-Time Polymerase Chain Reaction.

KIT COMPONENT

COVID-19 Plus RealAmp Kit	100 tests/Kit
COVID-19 Plus Reaction Mixture[1)]	1,050 µL
COVID-19 Plus Probe Mixture[2)]	550 µL
COVID-19 Plus Positive Control[3)]	50 µL
COVID-19 Plus Negative Control[4)]	50 µL

1) COVID-19 Plus Reaction Mixture: Containing Tris-HCl, $MgCl_2$ and dNTPs
2) COVID-19 Plus Probe Mixture: Primer pairs and probes for amplification and detection of each target
3) COVID-19 Plus Positive Control; Positive control for targets
4) COVID-19 Plus Negative Control; Ultrapure quality water, PCR-grade

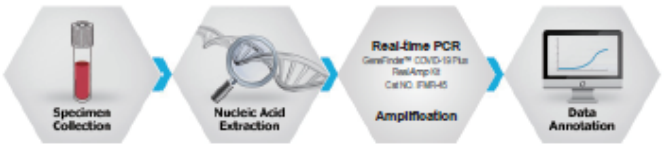
PREREQUISITE

- Applied Biosystems 7500/7500Fast and CFX96 Real-time PCR Instrument system
- Pipettes and Pipettes tips with aerosol barrier
- Vortex mixer
- Centrifuge with rotor for microtiter plates
- Disposable powder-free gloves.

WARNING AND PRECAUTION

- This product is designed for *In vitro diagnostics* (IVD).
- The test has to be performed by well-trained and qualified personnel.
- Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.
- Unnecessary repeated freezing and thawing will be occurred inaccurate results.
- Do not mix reagent from different batches of the kit.
- Do not modify the reagent/sample volume used in the test or use in a wrong way which is not recommended.

WORK FLOW



PROTOCOL

A. Specimen

This product is must be used with viral RNA samples extracted from human respiratory specimens such as alveolar lavage fluid, nasopharyngeal swabs (NPS), sputum etc.

PROTOCOL

B. RNA Extraction

It is recommended to use commercialized extraction kit such as QIAamp Viral RNA mini kit (Qiagen).

C. Reagent Preparation

Before setting up PCR, all components need to be thawed, gently mixed and centrifuged briefly to collect solution at the bottom.

1. Mix 10 µL of COVID-19 Plus Reaction Mixture and 5 µL of COVID-19 Plus Probe Mixture to prepare master mixture per each reaction (refer to the below). Prepare enough volume of master mixture for all the reactions plus extra to prevent pipetting error.
2. After mixing well, place 15 µL of master mixture into 96-well plate or PCR tube.
3. Add 5 µL of extracted RNA sample into 96-well plate or tube, then mix all components by pipetting. Proceed in the same way with other RNA samples, Positive Control and Negative Control.
4. Accurately close the tube with the cap or seal the 96-well plate.
5. Transfer the tubes or 96-well plate for test into the Real-time PCR and start for the amplification.

Component	Per reaction (µL)
COVID-19 Plus Reaction Mixture	10
COVID-19 Plus Probe Mixture	5
Sample or PC[1] or NC[2]	5
Total volume	20

[1], PC, Positive Control; [2], NC, Negative Control

D. Setting of Real-time PCR

This product is validated on ABI7500/7500Fast and CFX96 Real-time PCR instrument system.

1. Referring to the instrument manual, set on the dedicated software for the parameters of thermal cycle.
2. Set up the PCR program and fluorescence as following, and then click the start "Run" button.

PCR program			
Cycle		**Temp.**	**Time**
Segment 1	1 cycle	50 °C	20 min
	1 cycle	95 °C	5 min
Segment 2	45 cycles	95 °C	15 sec
		58 °C*	60 sec

* Select "Collect Data"

Fluorescence Setting	
Target	**Fluorophore**
RdRp gene	FAM
N gene	JOE (ABI) / VIC (CFX96)
E gene	Texas Red
IC	Cy5
NO QUENCHER, Reference dye None	

E. Analysis Setting

The values of fluorescence emitted by the specific probes and by the specific internal control probe in amplification reactions should be analyzed by the instrument software.

1. Click Analysis mode after completion and choose analysis setting from Amplification Plot.
2. Click "Edit Default Settings to set threshold values as shown below.

Target	Threshold		Baseline	
	ABI	**CFX96**	**Begin**	**End**
RdRp gene	30,000	300	3	15
N gene	30,000	300	3	15
E gene	30,000	300	3	15
IC	10,000	100	3	15

F. Result Interpretation

#	Ct range				Result
	RdRp (FAM)	E (Texas Red)	N (JOE)	IC (Cy5)	
1	Positive	Positive	Positive	Positive*	SARS-CoV-2 Positive
2	Positive	Positive	Negative	Positive	
3	Positive	Negative	Positive	Positive	
4	Positive	Negative	Negative	Positive	SARS-CoV-2 Positive
5	Negative	Positive	Positive	Positive	SARS-CoV-2 Positive
6	Negative	Negative	Positive	Positive	SARS-CoV-2 Positive
7	Negative	Positive	Negative	Positive	SARS-CoV-2 Presumptive Positive
8	Negative	Negative	Negative	Positive	Negative
9	Negative	Negative	Negative	Negative	Invalid (re-test)

Quality Control

- Positive Control and Negative Ct range should be as below:

#	FAM	JOE/VIC	Texas Red	Cy5
PC	≤35	≤35	≤35	≤35
NC	U.D	U.D	U.D	U.D

PERFORMANCE

Criteria	Result
Analytical Specificity	14 DNA/RNA samples were tested on the GeneFinder™ COVID-19 Plus Real*Amp* Kit in order to evaluate the possibility of cross-reactivity. 14 DNA/RNA samples which have no concern with the detection target of the kit were negative. * Cross reactivity : 100% Specificity
Analytical Sensitivity	Serial dilutions (10, 5, 1 copies/test) of COVID-19 RNA were tested. * Analytical Sensitivity : 1) RdRp gene: 10 copies/test 2) N gene: 10 copies/test 3) E gene: 10 copies/test
Repeatability	Repeatability was confirmed with identical standard substances at different conditions; different LOT, place, time and testers by 3 batch testing. Criteria of repeatability was CV < 5% of Ct Value.
Freeze/Thaw Safety	Freeze/Thaw safety of GeneFinder™ COVID-19 Plus Real*Amp* Kit was confirmed by 12 times of Freeze/Thaw repeat test. Criteria of safety was CV < 5% of Ct Value.

TROUBLE SHOOTING

Problem	Possible Cause	Recommendation
No signal in all samples including positive control	Error in Master mixture preparation	Check the dispensing volume during preparation of master mixture
	Inhibitors added	Repeat the extraction step with new sample
	Probe degradation	Use a new probe reagent
	Positive Control degradation	Use a new positive control
	Omitted components	Verify each component, repeat the RT-PCR mixture preparation
	Instrument setting error	Check the position setting for the positive control on the instruments. Check the Thermal cycle settings on sample instrument
Diverse intensity of fluorescent signals	Pipetting error	Make sure that the equal volume of reactants is added in each tube or plate
	Contamination in the outer surface of PCR tubes or Plate	Wear gloves during the experiment
Weak or no fluorescent signal in samples only	Poor RNA quality	Use recommended kit for RNA extraction and store extracted RNA at -70℃
	Insufficient volume of RNA	Repeat PCR reaction with correct volume of RNA