GeneFinder™
" SMART Solution for Better Life"

ISO 13485 Certified

GeneFinder™ COVID-19 Plus Real*Amp* Kit

REF IFMR-45 Σ 100 tests/Kit

Store at - 20°C or below.
Shelf life is 16 months after manufacturing.

CE *CE-IVD*
For ABI7500, ABI7500 Fast, CFX96, QuantStudio 5, qTOWER³ and Gentier 96E

INTENDED USE

GeneFinder™ COVID-19 Plus Real*Amp* 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 Real*Amp* 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.

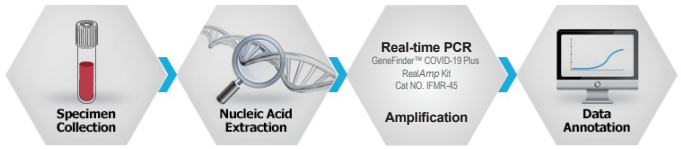
PREREQUISITE

- Applied Biosystems 7500 (ABI), Applied Biosystems 7500 Fast (ABF), CFX96 (CFX), QuantStudio 5 (QS5), qTOWER³ (QT3) and Gentier 96E (GTE) 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 Nasopharyngeal & Oropharyngeal (Throat) swab, Sputum and Bronchoalveolar Lavage Fluid (BAL)

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 the 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

(continued on reverse page)

D. Setting of Real-time PCR

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	Ramping rate** (qTOWER³)
1 cycle	50 ℃	20 min	4 ℃/S
1 cycle	95 ℃	5 min	4 ℃/S
45 cycles	95 ℃	15 sec	4 ℃/S
	58 ℃*	60 sec	2 ℃/S

* Select "Collect Data"
** For qTOWER³, set the Ramping rate as follows.

Fluorescence Setting

Target	ABI/ABF	QS5	CFX/GTE	QT3	
				Reporter dye	Gain
RdRp gene	FAM	FAM	FAM	FAM	3
N gene	JOE	VIC	VIC	HEX-3	5
E gene	Texas Red	ROX	Texas Red	Texas Red	5
IC	Cy5	Cy5	Cy5	Quasar 670	5
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/ABF	QS5	CFX	GTE	QT3	Begin	End
RdRp gene	30,000	15,000	300	1,000	3	3	15
N gene	30,000	15,000	300	1,000	3	3	15
E gene	30,000	15,000	300	1,000	3	3	15
IC	10,000	5,000	100	300	3	3	15

3. For qTOWER³, set the color compensation setting to Standard 1.

F. Result Interpretation

#	RdRp	N	E	IC	Assay Result
1	**≤40**	**≤40**	**≤40**	≤35	COVID-19 Positive
2	**≤40**	**≤40**	U.D*	≤35	COVID-19 Positive
3	**≤40**	U.D	**≤40**	≤35	COVID-19 Positive
4	**≤40**	U.D	U.D	≤35	COVID-19 Positive
5	U.D	**≤40**	**≤40**	≤35	COVID-19 Positive
6	U.D	**≤40**	U.D	≤35	COVID-19 Positive
7	U.D	U.D	**≤40**	≤35	Beta coronavirus
8	U.D	U.D	U.D	≤35	Negative
9	U.D	U.D	U.D	U.D	Invalid (re-test)

*Undetermined (or Not applicable (N/A) or No Ct or '-')

QUALITY CONTROL

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

#	RdRp gene	N gene	E gene	IC
PC	≤22	≤22	≤22	≤21
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 (1000, 100, 10, 1 copies/test) of COVID-19 RNA (3 batches, 24 times of repeat test each) 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°C
	Insufficient volume of RNA	Repeat PCR reaction with correct volume of RNA

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