GeneFinder™
" SMART Solution for Better Life"

OHC OSANG HEALTHCARE

GeneFinder™ COVID-19/Flu A&B Real*Amp* Kit

REF IFMR-57 Σ 100 tests/Kit

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

CE *CE-IVD*
For ABI 7500/7500 Fast, CFX96, Quantstudio 5, Gentier 96E, and qTOWER³

INTENDED USE

GeneFinder™ COVID-19/Flu A&B RealAmp Kit is a real-time reverse transcriptase polymerase chain reaction (PCR) test intended for the qualitative detection of nucleic acid of the SARS-CoV-2 (RdRp,N gene) or/and Influenza A&B in human respiratory specimens collected by a healthcare provider, from patients who are suspected of COVID-19 or/and Influenza A&B infection.

KIT COMPONENT

COVID-19/Flu A&B Real*Amp* Kit	100 tests / Kit
COVID-19/Flu A&B Reaction Mixture [1)]	1,000 µL
COVID-19/Flu A&B Probe Mixture [2)]	500 µL
COVID-19/Flu A&B Positive Control [3)]	50 µL
COVID-19/Flu A&B Negative Control [4)]	50 µL

[1)] COVID-19/Flu A&B Reaction Mixture: Containing RTase (Reverse Transcriptase), Taq polymerase, Tris-Cl, $MgCl_2$ and dNTPs.
[2)] COVID-19/Flu A&B Probe Mixture: Primer pairs and probes for amplification and detection of each target.
[3)] COVID-19/Flu A&B Positive Control: Positive control for each target.
[4)] COVID-19/Flu A&B Negative Control: Ultrapure quality water, PCR-grade.

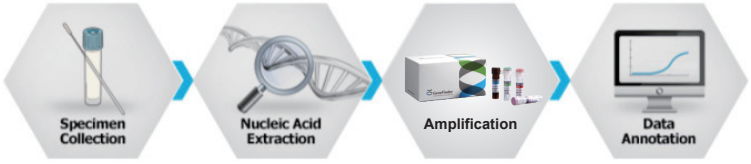
PREREQUISITE

- Applied Biosystems 7500/7500 Fast (ABI/ABF), CFX96 (CFX), QuantStudio 5 (QS5), Gentier 96E (GTE) and qTOWER³ (QT3) Real-time PCR Instrument system.
- Pipettes and Pipettes tips with aerosol barrier.
- Vortex mixer.
- Centrifuge.
- 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 lead to 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 must be used with viral RNA samples extracted from human respiratory specimens such as Nasopharyngeal aspirate, Nasopharyngeal & Oropharyngeal (Throat) swab, and Sputum etc.

B. RNA Extraction

It is recommended to use commercialized extraction kit such as QIAamp Viral RNA mini kit (Qiagen) and Magna Pure system (Roche).

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/Flu A&B Reaction Mixture, 5 µL of COVID-19/Flu A&B Probe Mixture to prepare master mixture per each reaction (refer to the chart below). Prepare enough volume of master mixture for all of the reactions. Prepare extra mixture to prevent pipetting error.
2. After mixing well, place 15 µL of master mixture into a 96-well plate or PCR tube.
3. Add 5 µL of extracted RNA sample into 96-well plate or tube, then mix all of the 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/Flu A&B Reaction Mixture	10
COVID-19/Flu A&B 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

This product is validated on ABI7500/7500 Fast, CFX96, QuantStudio 5, Gentier 96E, and qTOWER^3 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	Ramping rate** (qTOWER^3)
Segment 1	1 cycle	50 °C	20 min	4 °C/S
	1 cycle	95 °C	10 min	4 °C/S
Segment 2	45 cycles	95 °C	15 sec	4 °C/S
		58 °C*	60 sec	2 °C/S

* Select "Collect Data" or "plate Read"
** For qTOWER^3, set the Ramping rate as follows.

Fluorescence Setting

Target	ABI/ABF	QS5	CFX/GTE	qTOWER^3 (Gain value)
Flu A (Matrix)	FAM	FAM	FAM	FAM (3)
Flu B (Nucleoprotein)	Texas Red	ROX	Texas Red	Texas Red (5)
COVID-19 (RdRp, N)	JOE	VIC	VIC	HEX-3 (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.

Fluorophore	Threshold				Baseline	
	ABI / QS5	CFX	GTE	qTOWER^3*	Begin	End
FAM	30,000	500	1,000	3	3	15
Texas Red / ROX	30,000	500	1,000	3	3	15
JOE/VIC/HEX-3	30,000	500	1,000	3	3	15
Cy5/Quasar670	10,000	100	300	3	3	15

*For qTOWER^3, set the color compensation setting to Standard 1.

F. Result Interpretation

#	Flu A	Flu B	COVID-19	IC	Assay Result
1	**≤40**	**≤40**	**≤40**	≤35	Influenza A (+) Influenza B (+) COVID-19 (+)
2	**≤40**	**≤40**	U.D	≤35	Influenza A (+) Influenza B (+)
3	**≤40**	U.D	**≤40**	≤35	Influenza A (+) COVID-19 (+)
4	**≤40**	U.D	U.D	≤35	Influenza A (+)
5	U.D	**≤40**	**≤40**	≤35	Influenza B (+) COVID-19 (+)
6	U.D	**≤40**	U.D	≤35	Influenza B (+)
7	U.D	U.D	**≤40**	≤35	COVID-19 (+)
8	U.D	U.D	U.D	≤35	Negative
9	U.D	U.D	U.D	U.D	Invalid (re-test)

Note : When the target RNA is detected in a sample amplification reaction, the Internal control (IC) may give the result as Ct Not applicable (N/A) or Undetermined (U/D). In fact, the low-efficiency amplification reaction for the internal control may be displaced by competition from the high-efficiency amplification reaction for Target gene. In such a case, it shall be determined as positive.

QUALITY CONTROL

• PC and NC Ct range should be as below:

#	FAM	JOE/VIC/HEX-3	Texas Red/ROX	Cy5/Quasar 670
PC	≤35	≤35	≤35	≤35
NC	U.D or N/A	U.D or N/A	U.D or N/A	U.D or N/A

PERFORMANCE

Criteria	Result
Analytical Specificity	25 DNA/RNA samples were tested on the GeneFinder™ COVID-19/Flu A&B Real*Amp* Kit in order to evaluate the possibility of cross-reactivity. 25 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, 2, 0.2 copies/ul) of COVID-19/Flu A&B RNA (3 batches, 24 times of repeat test each) were tested. * Analytical Sensitivity : 1) Influenza A : 5 copies/ul 2) Influenza B : 5 copies/ul 3) COVID-19 : 2 copies/ul
Reproducibility	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/Flu A&B Real*Amp* Kit was confirmed 12 times by 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

OSANG Healthcare Co., Ltd. 132, Anyangcheondong-ro, Dongan-gu, Anyang-si, Gyeonggi-do, 14040, Korea
For technical support, Tel. +82-31-460-0300 Fax. +82-31-460-9933 Web. http://www.osanghc.com