GeneFinder™ COVID-19 PLUS RealAmp Kit

Instructions for Use

REF IFMR-45

IVD CE

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In Vitro Diagnostic

1. Intended Use

GeneFinder ™ COVID-19 PLUS RealAmp Kit is used for detection of COVID-19 (COIVD-19) virus through reverse Transcription and Real-Time Polymerase Chain Reaction from RNA extracted from Respiratory specimens such as Alveolar lavage fluid, throat swab, sputum. This product can qualitatively detect COVID-19 using Polymerase Chain Reaction.

2. Principle of the Assay

One-Step Reverse Transcription Real-Time polymerase chain reaction is used to confirm the presence of COVID19 by amplification of RdRp, E and N gene

This product is a In vitro diagnostics (IVD) and is used by professionals in hospitals and laboratories.

3. Kit Contents

Reagents / Materials	100 tests/Kit
COVID-19 PLUS Reaction Mixture	1,050 ul
COVID-19 PLUS Probe Mixture	550 ul
COVID-19 PLUS Positive Control	50 ul
COVID-19 PLUS Negative Control	50 ul

4. Storage and Handing Requirements

- All components of the kit should be stored at -20°C or below and kept stable until the expiry date stated on the label.
- The COVID-19 PLUS Probe Mixture must be stored below -20°C and in the dark.
- Expires 12 months after date of manufacture. Do not use after expiration date.
- Expires 6 months after opening the kit. Do not use after use life time.
- Store the rest of the kit below -20°C.
- If the kit is defective, do not use.
- Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.

Note: Inaccurate results can be obtained if the kit is stored at room temperature for a long period of time.

Note: Unnecessary repeated freezing and thawing lead to inaccurate results.

5. Product Description

1) **COVID-19 PLUS Reaction Mixture**
   COVID-19 PLUS reaction mixture with reagents for reverse transcription and amplification

2) **COVID-19 PLUS Probe Mixture**
   Buffer solution with specific primers and probes which conjugate with nucleic acids of COVID-19 virus and internal control

3) **COVID-19 PLUS Positive Control**
   Positive Control determines for presence of errors/contamination during the test.
   Caution. Care should also be taken to avoid cross-contamination of other samples when adding Positive Control.

4) **COVID-19 PLUS Negative Control**

To confirm the absence of contamination, negative control reaction should be included at every run as it indicates that reagents have not been contaminated.

* The product allows the accomplishment of 100 tests, including controls.

6. Required materials

6.1. Provided in the product

(100 tests / Kit)

Label	Cap color	Storage	Quantity
COVID-19 PLUS Reaction Mixture	Purple	-20℃	1 x 1,050 ul
COVID-19 PLUS Probe Mixture	Brown	-20℃	1 x 550 ul
COVID-19 PLUS Positive Control	Red	-20℃	1 x 50 ul
COVID-19 PLUS Negative Control	Green	-20℃	1 x 50 ul

6.2. Required but not provided in the product

- Applied Biosystems® 7500 / 7500 Fast Real Time PCR Instrument System and CFX96 real time PCR system.
- Pipettes (1- 20 µl, 20-200 µl, 200-1,000 µl)
- Pipettes tips with aerosol barrier (RNase, DNase-free)
- Powder-free gloves (disposable)
- Vortex mixer or equivalent
- 1.5 ml tube
- PCR tube or 96 well plate
- Bench microcentrifuge
- RNA isolation kit (Use of QIAamp Viral RNA Mini Kit (Cat. # 52904, Qiagen) is recommended or commercial kits)

7. Warning and Precaution

The GeneFinder™ COVID-19 Plus Real*Amp* Kit is designed for ***In vitro diagnostics***

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General warnings and precautions

- Read the instructions in the package carefully before processing samples.
- Use 0.5% v / v sodium hypochlorite or another disinfectant to clean and disinfect the area around the sample.
- Decontaminate and dispose of all specimens, reagents and other potentially contaminated materials in accordance with local regulations.
- Use universal precautions when performing the assay. Handle samples as if capable of transmitting infection.
- Wear personal protective apparel, including disposable gloves, throughout the assay procedure. Thoroughly wash hands after removing gloves, and dispose of gloves as biohazardous wastes.
- The material that come into contact with the biological samples must be autoclaved for one hour at 120℃ before disposal.
- Do not eat, drink, smoke, or apply cosmetics in areas where reagents of samples are handled.
- Do not pipet by mouth.
- Do not use a kit after its expiration date.

- Use aerosol-resistant pipette tips and use a new tip every time a volume is dispensed.
- Store the reagents recommended temperature.
- Do not mix reagent from different batches of the kit.
- Store the kit away from any source of contaminating DNA, especially amplified nucleic acid. - Use sterile disposable laboratory materials and do not re-use the tubes and tips.
- Alterations in the physical appearance of kit components may indicate instability or deterioration.
- Use all pipetting devices and instruments with care and follow the manufacturer's instructions for calibration and quality control.
- Do not modify the reagent/sample volume used in the test or use in a wrong way which is not recommended.
- Store COVID-19 PLUS Probe Mixture at -20℃ in a dark place.

8. Procedure

8.1 Preparation of sample

The GeneFinder™ COVID-19 PLUS Real*Amp* Kit must be used with RNA extracted from Alveolar lavage fluid, throat swab, and sputum samples. RNA extraction is recommended from sample as soon as possible for accurate experiments.

8.1 RNA Extraction

Commercialized extraction kit should be used for collection of RNA Extracted samples. QIAamp viral RNA Mini Kit (Qiagen, Germany, Cat. # 52904) is recommended for extraction. Please carry out RNA extraction according to the manufacturer's instructions.

Extracted RNA samples are more vulnerable than DNA that it is suggested to avoid repeated freezing and thawing and to keep at -70°C.
When extracting RNA, be sure to extract it according to the manufacturer's instructions.

8.2 Preparation of reagents

Thaw all components thoroughly at room temperature before using. Mix gently, spin down the contents for 5 seconds, and then test it immediately..

1. Mix 10 µl of COVID-19 PLUS Reaction Mixture, 5 µl of COVID-19 PLUS Probe Mixture to prepare RTPCR Master mixture as described in the following table (Table 1). Prepare enough volume of Master mixture for all the reactions plus extra amounts to prevent possible pipetting error.

   Note: Total Master mixture number
   = n sample + 1 positive control + 1 negative control + 1 extra

Number of Samples / Solution	1 TEST	3 TEST	5 TEST	Total volume of Master Mixture
COVID-19 PLUS Reaction Mixture	10 ul	30 ul	50 ul	10x(n+3)
COVID-19 PLUS Probe Mixture	5 ul	15 ul	25 ul	5x(n+3)
Total (COVID-19 PLUS Master Mixture)	15 ul	45 ul	75 ul	20x(n+3)

Table 1. Master Mixture preparation

Important: Adequate controls should be used in each run to ensure reliable results.

2. Place 15 µl of RT-PCR Master mixture into each PCR tube or optical 96 well plate ***Note:*** To avoid any bubbles, do not vortex the tubes at this step.

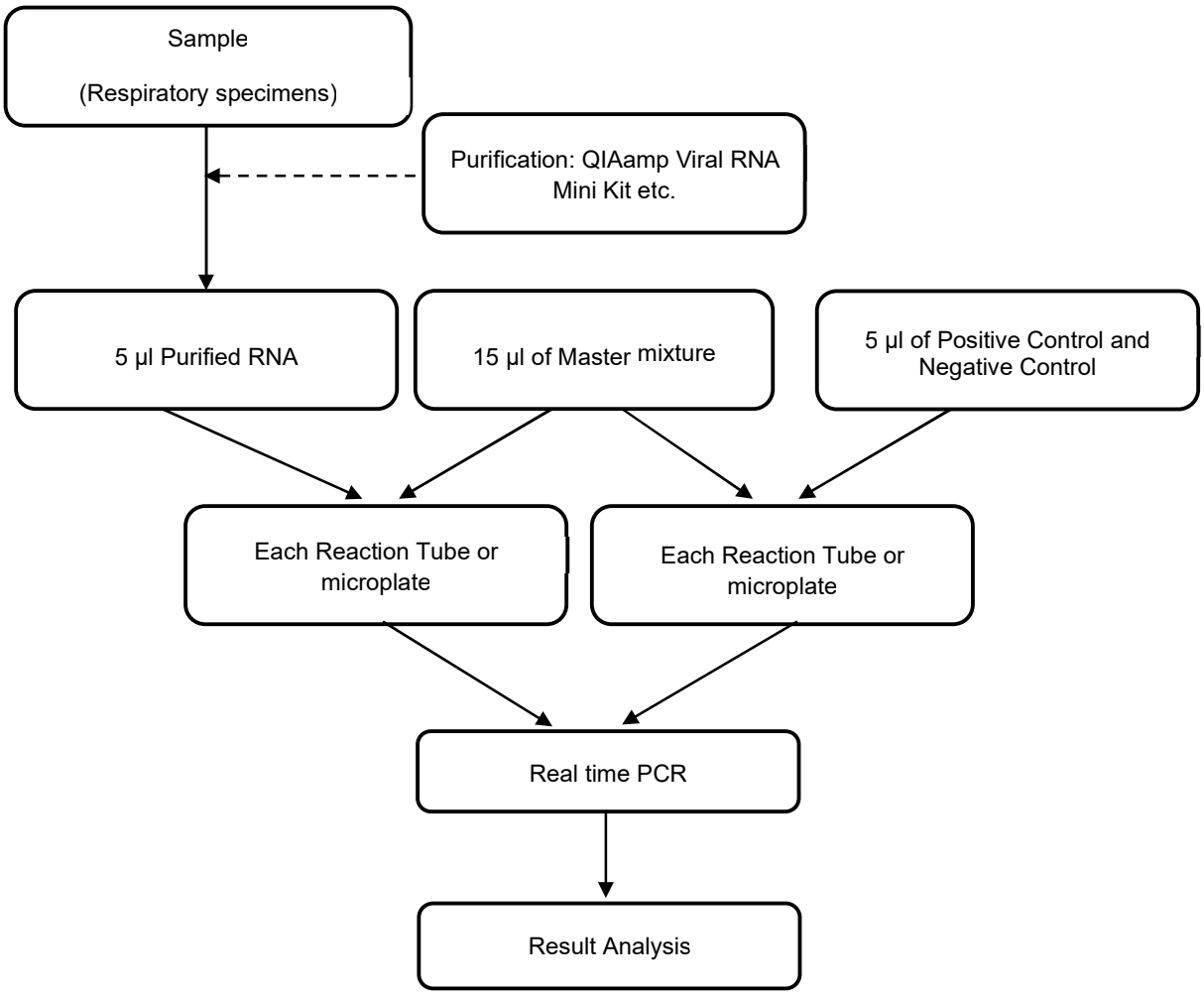
3. Add each of 5 µl of sample RNA into the corresponding PCR tube/well-plate for amplification and mix them with pipetting

4. Place 5 µl of Positive Control and Negative Control into the each PCR tube or well-plate in the same way.
   Note: Total Reaction volume is 20 µl per sample.
   Note: Insufficient mixing of the Master mixture may lead to inaccurate result..

5. Transfer PCR tube or well-plate for testing into the real-time thermal cycler and start the thermal cycle for the amplification.

Figure 1. Schematic workflow for test

8.3 Setting of the RealTime Amplificatino



Prior to amplification, operate PCR instrument according to the manufacturer's manual.

※ Real-Time PCR condition

Step		Temperature	Time	Cycles
1	Reverse Transcription	50℃	20 min	1 cycle
2	Pre-denaturation	95℃	5 min	1 cycle
3	Denaturation	95℃	15 sec	45 cycles
	Annealing*	58℃	60 sec	

*Collection of data

※ Fluorescence setting

Target	Fluorescence
RdRp gene	FAM
E gene	Texas Red
N gene	JOE (ABI) / VIC (CFX96)
Internal Control	Cy5

8.4 Data analysis

1. Select Amplification Plot at Analysis Mode.
2. Select Analysis Settings.
3. Set Threshold Values, Baseline start and end values.

Target	Threshold		Baseline	
	ABI 7500/ ABI 7500 fast	CFX 96	Begin	End
RdRp gene (FAM)	30,000	300	3	15
E gene (Texas Red)	30,000	300	3	15
N gene (JOE)	30,000	300	3	15
Internal Control (Cy5)	10,000	100	3	15

9. Result

Result Interpretation.

#	Ct range				Result
	RdRp (FAM)	**E** (Texas Red)	**N** (JOE)	**IC** (Cy5)	
1	≤43	≤43	≤43	≤35*	COVID-19 Positive
2	≤43	≤43	U.D	≤35	
3	≤43	U.D	≤43	≤35	
4	≤43	U.D	U.D	≤35	Repeat the test (COVID-19 Positive if RdRp≤43)
5	U.D	≤43	≤43	≤35	Repeat the test (COVID-19 Positive if E and N≤43)
6	U.D	U.D	≤43	≤35	Repeat the test (COVID-19 Positive if N≤43)
7	U.D	≤43	U.D	≤35	Beta coronavirus
8	U.D	U.D	U.D	≤35	Negative
9	U.D	U.D	U.D	U.D	Invalid (re-test)

Note

- *When target RNA is detected in a sample amplification reaction, Internal control (IC) may give the result as Ct Not applicable (N/A). In fact, low-efficiency amplification reaction for internal control may be displaced by competition from high-efficiency amplification reaction for Target gene. In such a case, it shall be determined as positive.
- If test result is not valid as follows, it is recommended to retest.

① In case of Ct value of Internal control is Not applicable

② In case of Invalid result.

10. Quality Control

Validating the whole analysis procedure (of each extraction and amplification session by processing a negative tested sample and a positive tested sample or a calibrated reference material) is recommended.

11. Procedure Limitation

- The users must be trained and familiar with this technology prior to the use of this device.
- Any diagnostic results generated must be interpreted in conjunction with other clinical or laboratory findings. It is the user's responsibility to validate system performance for any procedures used in their laboratory which are not covered by the OSANG Healthcare performance studies.
- Use this product only with RNA extracted from the following human biological samples: Alveolar lavage fluid, throat swab, sputum
- A negative result does not exclude the possibility of infection, because results are dependent on appropriate specimen collection and absence of inhibitors. The presence of PCR inhibitors may cause invalid results with this product..

12. Trouble shooting

Problems	Possible Causes	Recommendation
If no fluorescent signal is detected in all samples, including positive control	Error in the preparation of the master mixture	Check the volumes of reagent dispensed during preparation of the master mixture
	Inhibitors added	Take care when RNA is extracted and repeat the extraction step with new sample
	Probe degradation	Use a new probe aliquot
	Positive control degradation	Use a new aliquot of Positive control
	Omitted components	Verify each component and repeat the PCR mixture preparation
	Instrument setting error	Check position settings for the positive control on the instrument Check the thermal cycle settings on the instrument
If the fluorescent signal is detected in negative control reaction except CY 5.	Carry-over contamination	Take care when dispensing samples, negative controls, and positive controls on the instrument Always change tips between one sample and another
	Microplate/ tube Error	Be careful not to spill the contents of the tube or plate.
	Tube cap badly sealed	Take care when sealing the tube cap
	Contamination of the amplification mix	Use a new aliquot of amplification mix
	Contamination of the extraction/preparation area for amplification reactions	Clean surfaces and instruments with aqueous detergents, wash lab coats replace test tubes and tips in use
If the fluorescent intensity is weak	Poor quality of RNA samples	Extract RNA from samples using the recommended kit, and store the extracted RNA at -70℃

or does not appear only in the unknown samples	Not enough volume of RNA samples added	Repeat the PCR reaction using the correct volume of RNA samples
If the fluorescent intensity is weak or does not appear in the positive control	Probe degradation	Use a new probe aliquot Test should be done with a new kit
If the diverse intensity of fluorescent signals appear	Pipetting error	Make sure that an equal volume of reactants is added in each tube
	Contamination in the outer surface of PCR tubes and plate	Wear gloves during the experiment

13. Performance characteristics

Analytical Sensitivity : LOD

Analytical sensitivity with Target RNA was performed with GeneFinder™ COVID-19 PLUS RealAmp Kit. As a result of experiments at various concentrations, the analytical sensitivity of the product was confirmed as **10 copies / reaction** for all target genes (RdRp, E, N gene).

Analytical Specificity : Cross Reactivity

The cross reactivity tests were performed using COVID-19 Standard materials and RNA of 14 negative reference materials; None of negative reference materials were detected in any of the test performed.

#	Name
1	Influenza A (H1N1/09)
2	Influenza A (H3N2)
3	Influenza A (H5N1)
4	Influenza B
5	Rhinovirus
6	Respiratory syncytial virus (A/B)
7	Parainfluenza 1 virus
8	Parainfluenza 2 virus
9	Parainfluenza 3 virus
10	Parainfluenza 4 virus
11	Adenovirus
12	Human Bocavirus
13	Measles virus
14	Mycoplasma spp.

Reproducibility (Between Lots, operators, and places)

Reproducibility between lots, inspectors, and test sites with different manufacturing dates for the same concentration of standard material was found to be within the CV% titration range (<5 CV%) and showed reproducibility.

Repeatability

Repeated every 2 days, the test is carried out by replication; all results are taken as the reference value (5% sustained CV basis).

Interference

Mucin, NaCl, Blood, Respiratory syncytial virus A, and PBS were repeated four times for each of high, medium and low concentrations. As a result of testing with RNA extracted from swab containing interfering substances, it was confirmed that some substances above a certain concentration act as interfering agents in reverse transcription and PCR reactions. At the appropriate concentration, all showed positive test results of 100% and confirmed that they were not affected by the interference materials.

14. Symbols used on Labels

Lot or batch number


Caution

Catalogue number

Store below temperature shown

In Vitro Diagnostic Medical Device


Expiry date


Consult Instruction For Use

Manufacturer 

15. Reference

1. WHO COVID-19 report 2020