

## 1. Description

1copy™ COVID-19 qPCR Kit provides the fast and accurate testing solution for COVID-19, specifically targeting the E gene for beta coronavirus and the RdRp gene for COVID-19 in sputum, nasopharyngeal swab and oropharyngeal swab.

Our COVID-19 Real-time PCR assay is based on the WHO & KCDC reference method and it has been carried out the in silico analysis for all registered COVID-19 sequence database.

## 2. Intended Use

1copy™ COVID-19 qPCR Kit is an In-Vitro Diagnostic medical device for qualitative analysis of E gene and RdRp gene for coronavirus (COVID-19) in extracted RNA from sputum, nasopharyngeal swab and oropharyngeal swab of patients with suspected respiratory infections.

## 3. Materials Provided

Kit contents	Cap color	Volume (100 Test)
Master mix	Red	1000 µl x 2
Primer/Probe mix 1(E gene)	Brown (Amber tube)	100 µl
Primer/Probe mix 2(RdRp gene)	Brown (Amber tube)	100 µl
Control 1 (E gene)	Yellow	100 µl
Control 2 (RdRp gene)	Yellow	100 µl
DEPC Water	Clear	1000 µl

## 4. Materials Required but Not Provided

- ① RNase/DNase free consumables (disposable latex or vinyl gloves)
- ② Filter tips
- ③ 0.5ml or 0.2ml PCR tubes or 96-well PCR plates
- ④ 1.5ml micro tubes
- ⑤ Sealing film
- ⑥ Ice or cooling/cold block
- ⑦ Microliter pipettes (1~10µl, 10~100µl, 100~1000µl)
- ⑧ Mini centrifuge (0.2ml/0.5ml tubes, 10,000 rpm) or Benchtop centrifuge with rotor for 0.2ml /0.5ml reaction tubes (capable of attaining 10,000 rpm), vortexer
- ⑨ Real-time PCR instrument
- ⑩ Reagents or Kit for RNA extraction

- Prepare RNA specimens using RNA extraction Kit or manual method for RNA extraction.

## 5. Warnings and Precautions

1. 1copy™ COVID-19 qPCR Kit is for In Vitro Diagnostic use only.
2. This product is intended for professional use and should only

be used by qualified and experienced inspectors for us in clinical specimens and molecular biology experiments.

3. Do not use expired components.
4. Wear appropriate protective clothing, disposable gloves and protective gloves.
5. Use filter pipette tips to avoid contamination.
6. Do not mix reagents from different lots of 1copy™ COVID-19 qPCR Kit.
7. Minimize the temperature difference of the components.  
Thaw necessary components just before using and promptly place back in freezer after use.
8. Use thawed contents after gently mix and spin down.
9. Prepare mixture of qPCR within a cooling/cold block or on ice.
10. Discard unused reagents, waste and control according to laboratory safety rules and guidelines.
11. In case of contact with eyes, rinse immediately with water.
12. Do not freeze/thaw all components of 1copy™ COVID-19 qPCR Kit more than 5 times.
13. We guarantee the optimal performances when user follow the instructions given in this manual.
14. Even if the test results of this product are 'positive', it should be interpreted by an experienced specialist and review of various results such as the patient's symptoms.
15. Even if the test results of this product are 'negative', it should be interpreted by an experienced specialist and review of various results such as the patient's symptoms without excluding infection.

## 6. Reagent storage and handling

1. Store the Kit below -20°C.
2. Expiration date for Kit is indicated on the packing box. Freezing and thawing is limited to 5 times.
3. Minimize the temperature difference of the components.  
Thaw necessary components just before using and promptly place back in freezer after use.

## 7. Specimen collection, storage and preparation

1. The collection, storage and preparation of specimens should be guided by KCDC.

	Specimen type	Container and capacity
1	Lower respiratory track • Sputum	• (Container) Sterilized 50ml Tube • (Sample volume) More than 3ml
2	Upper respiratory track • Nasopharyngeal swab • Oropharyngeal swab	• (Container) Simultaneous collection of nasopharyngeal and oropharyngeal swab on one VTM medium
3	Etc • Blood • Stool/ Urine	• (Container) SST 5~10ml, 1ml for infants • (Container) Sterilized 50ml Tube
(Required specimen) Upper/ Lower respiratory (Selective specimen) Blood		

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\* Specimen collection method  
Reference: CDC, Interim Guidelines for Collecting, Handling and Testing Clinical Specimens from Persons Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV), 2020.2.2 ver

When collecting specimens, personal protective equipment such as N95 respirators, disposable gloves, gowns, goggles or face protection is mandatory.

## 2. Use RNA extracted from patient's specimen.

The quality of the assay is largely dependent on the quality of input RNA. So RNA preparation from patient samples should be performed using a validated extraction procedure.

For reagents required for RNA extraction, viral RNA extraction Kits from various manufactures are available.

### \* Recommended Kit

- Kogenebiotech  
(PowerPrep™ Viral DNA/RNA Extraction Kit, Cat. No. IE0007)
- QIAGEN  
(QIAamp DSP Viral RNA Mini Kit, Cat No. 52904)

## 8. Procedure

### 1. RT-qPCR preparation

- ① Make mixture in PCR tube according to below table.

- Recommended to use sample of RNA 5µl/reaction
- Use control 5µl/reaction
- Use negative control 5µl/reaction of the provided DEPC-DW

\* Number of reactions for each primer probe mix  
= total number of samples + 2

E gene primer Probe mix1 use + Control1 + Negative Control  
(number of samples) (E Gene)

RdRp gene primer Probe mix1 use + Control1 + Negative Control  
(number of samples) (E Gene)

Mixture Components	1 reaction (Total volume : 20 µl)
Master mix	10 µl
Primer Probe mix	1µl
RNA sample	5 µl
(In Control, instead of RNA sample)	(5 µl)
DEPC Water	Up to 20 µl

- ② Gently mix the mixture by vortexing and spin the tubes.
- ③ Place the samples on the 96 well PCR plate.
- ④ Cover wells the sealing film and spin the plate.
- ⑤ PCR reaction conditions follows as below.

Step	Temperature	Time	Cycle
RT	55°C	25 min	1
Incubation	95°C	5 min	1
Amplification	95°C	10 sec	45
	58°C *	30 sec	

\*Measure florescence at 58°C (FAM channel)

### 2. Software setting

#### \* Recommended Real-time PCR instruments

- Roche Light Cycler® 480  
(Roche, Product No. 05015278001),
- Rotor-Gene® Q 5plex HRM  
(Qiagen, Product No. 9001580),
- Applied Biosystems® Quantstudio5  
(Thermo Fisher Scientific, Product No. A28134),

#### \* Applied Biosystems® 7500 Real-Time PCR system

(Thermo Fisher Scientific, Product No. 4345241),

#### • CFX96™ Real-Time PCR Detection system

(Bio-Rad, Product No. 1854095-IVD)

- ① Run a software and click “New Experiment”
- ② Enter the reaction volume 20 µl and modify PCR reaction conditions as below.

Step	Temperature	Time	Cycle
RT	55°C	25 min	1
Incubation	95°C	5 min	1
Amplification	95°C	10 sec	45
	58°C *	30 sec	

\*Measure florescence at 58°C (FAM channel)

- ③ Select the type of measured FAM type of measured fluorescence as FAM.
- ④ Specify the sample, control, and negative control positions in the 96 well PCR plate layout on the program.
- ⑤ Select to start an experiment on the program.

## 9. Interpretation of results

Experimental results confirm the failure of the experiment through the result of control and negative control.

Positive/Negative(+/-) determination of each control, negative control, and sample result is determined by whether the curve of each result crosses the threshold line after applying the threshold value for each device. (+ if cross, otherwise -)

#### \*Threshold value for each equipment are as follows

- Light Cycler 480 = Whether to check Ct when setting
- Rotor-Gene Q 5plex HRM = 0.3
- Applied Biosystems® Quantstudio5 = 70,000  
“Abs Quant/2nd Derivative Max” is Analysis menu
- Applied Biosystems® 7500 = 250,000
- CFX96™ Real-Time PCR Detection system = 1,500

The interpretation of the results follows the table below.

#### \* E gene assay

Detection Channel	Control 1	Negative Control	Sample	Interpretation
FAM	+	-	+	beta coronavirus detected
	+	-	-	beta coronavirus not detected
	+	+	+/-	Test failed / Retest
	-	+	+/-	
	-	-	+/-	

#### \* RdRp gene assay

Detection Channel	Control 2	Negative Control	Sample	Interpretation
FAM	+	-	+	COVID-19 detected
	+	-	-	COVID-19 not detected
	+	+	+/-	Test failed / Retest
	-	+	+/-	
	-	-	+/-	



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