1copy™

1copy™ COVID-19 qPCR Multi Kit

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User Manual

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1. Description

1copy™ COVID-19 qPCR Multi 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 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 sillico analysis for all registered COVID-19 sequence database.

2. Intended Use

1copy™ COVID-19 qPCR Multi 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 nasopharyngeal swab and oropharyngeal swab of patients with suspected respiratory infections.

3. Materials Provided

Kit contents	Cap color	Volume (100 Test)
Master mix	Red	2 x 1000 µℓ
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 DW	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 Multi 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 Multi 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 Multi 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
Upper respiratory track	▪ Nasopharyngeal swab ▪ Oropharyngeal swab	▪ (Container) Simultaneous collection of nasopharyngeal and oropharyngeal swab on one VTM medium

* 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)
  · Gene All
  (Ribospin™ vRDII, Cat No.322-150)

8. Procedure

1. RT-qPCR preparation

① Make mixture in PCR tube according to below table.

E Mixture Components	1 Reaction (Total volume : 20 μℓ)
Master mix	10 μℓ
Primer Probe mix 1	1μℓ
RNA sample or Control (Control 1 and DEPC DW)	5 μℓ
DEPC DW	Up to 20 μℓ

RdRp Mixture Components	1 Reaction (Total volume : 20 μℓ)
Master mix	10 μℓ
Primer Probe mix 2	1μℓ
RNA sample or Control (Control 2 and DEPC DW)	5 μℓ
DEPC DW	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 Insert the plate into the PCR instrument.

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 start new experiment setting.
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
	60°C *	30 sec	

*Measure florescence at 60°C (FAM and Texas Red(or Red 610)channel)

③ Select the type of measurement fluorescence as FAM and Texas Red(or Red 610).
④ Specify the sample, control, and negative control positions in the 96 well PCR plate layout on the program.
⑤ Select to tart run an experiment on the program.

9. Interpretation of results

* Cut-off

Ct value	Result
≤40	Valid(+)
>40 or N/A	Invalid(-)

Ct values are calculated automatically by the instrument.
Ct values above 40 for FAM and Texas Red(or Red 610) signals may be the result of unspecific amplification (false positive).

The interpretation of the results follows the table below.

* E gene assay

Dye	Target
FAM	E gene - beta coronavirus
Texas Red(or Red 610)	IPC (Internal Positive Control)

* E gene assay Interpretation

Control 1 (FAM)	Negative Control	Sample (FAM)	IPC (Texas Red)	Interpretation
+	-	+	+/-	beta coronavirus detected
+	-	-	+	beta coronavirus not detected
+	-	-	-	Test failed / Retest
+	+	+/-	+/-	
-	+	+/-	+/-	
-	-	+/-	+/-	

* RdRp gene assay

Dye	Target
FAM	RdRp gene - COVID-19

* RdRp gene assay Interpretation

Control 2 (FAM)	Negative Control	Sample (FAM)	Interpretation
+	-	+	**COVID-19 detected**
+	-	-	COVID-19 not detected
+	+	+/-	Test failed / Retest
-	+	+/-	
-	-	+/-	

* Total result interpretation

Case	E gene	RdRp gene	Total result interpretation
1	detected	detected	COVID-19 detected
2	not detected	not detected	COVID-19 / beta coronavirus not detected
3	detected	not detected	beta coronavirus detected (Retest recommended)
4	not detected	detected	Test failed / Retest

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