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1copy™ COVID-19 qPCR 4plex Kit

Quick Guide

Cat no. M24MD100T

For *in vitro* diagnostic use
Prescription Use Only

※ Please refer to the Instructions for Use, which is available at http://www.1drop.co.kr/download for more details.

1. Intended Use

1copy™ COVID-19 qPCR 4plex Kit is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal, oropharyngeal, anterior nasal, mid-turbinate nasal swab specimens as well as nasopharyngeal wash/aspirate and nasal aspirate specimens collected from individuals suspected of COVID-19 by their healthcare providers.
Testing is limited to laboratories - certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests or similarly qualified non-U.S. laboratories.

Results are used to identify the presence of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results indicate presence of SARS-CoV-2 RNA, but clinical correlation with patient history and other diagnostic information are necessary to rule out a patient's infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

1copy™ COVID-19 qPCR 4plex Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

2. Kit Contents (Materials Provided)

Kit contents	Cap color	Volume (100 Tests)
Master mix	Red	1000 µℓ
Primer/Probe mix (E gene, RdRp gene, N gene, IPC)	Brown (Amber tube)	100 µℓ
Control (E gene, RdRp gene, N gene, IPC)	Yellow	100 µℓ
DEPC DW	Clear	1000 µℓ

※ Control is positive control.
※ DEPC DW (Diethylpyrocarbonate-treated water; nuclease-free water) is used as a negative control.

3. Materials Required but Not Provided

* Provided with the kit (please see kit contents, section 2)

- RNase/DNase free consumables (disposable latex or vinyl gloves)
- Filter tips
- 0.5 mℓ or 0.2 mℓ PCR tubes or 96-well PCR plates specified in PCR instrument manufacturer's instructions
- 1.5 mℓ micro tubes
- Sealing film
- Ice or cooling/cold block
- Microliter pipettes (1~10 µℓ, 10~100 µℓ, 100~1000 µℓ)
- Mini centrifuge (0.2 mℓ/0.5 mℓ tubes, 10,000 rpm) or Benchtop centrifuge (1.5 mℓ microcentrifuge and 96 well plate centrifuge) with rotor for 0.2 mℓ/0.5 mℓ reaction tubes (capable of attaining 10,000 rpm)
- Vortex mixer
- Sample collection and sample preservation buffer (Puritan UniTranz-RT 3 mℓ Filled Vial w/ Elongated & Ultrafine Flock Swabs (Cat No. UT-367))
- Real-time PCR instrument (See Section 4 below)
- QIAamp Viral RNA Mini Kit (Qiagen, Cat no.52904)
- Ethanol (96~100%)

4. Compatible Real-time PCR Instruments

- **Light Cycler 480** (Roche)
- **Rotor-Gene Q 5plex HRM** (Qiagen)
- **Applied Biosystems Quantstudio5** (Thermo Fisher Scientific)
- **Applied Biosystems 7500 Real-Time PCR Instrument System** (Thermo Fisher Scientific)
- **CFX96™ Real-Time PCR Detection System** (Bio-Rad)
- **qTOWER3 Real-Time PCR Thermal Cycler** (Analytik-jena)

5. Reagent Storage and Handling

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

6. Procedure

6.1 Specimen collection and storage

Inadequate specimen collection and improper specimen handling may yield a false result. Training in specimen collection is highly recommended due to the importance of specimen quality. CLSI MM13 (Clinical and Laboratory Standards Institute) may be referenced as an appropriate resource.
Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html
Refer to the CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19) www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html

Follow specimen collection devices manufacturer instructions for proper methods.
Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2-3 ㎖ of viral transport media or universal transport media.
Swab specimens for testing can be stored up to 72 hours at 2-8 °C, with long-term storage at -70 °C or below.

6.2 RNA extraction

* Validated Kit for extraction of nucleic acids
- QIAamp Viral RNA Mini Kit (Qiagen, Cat no.52904)

RNA extraction should be performed using QIAamp Viral RNA Mini Kit (Qiagen) according to the manufacturer's instructions and using the following specimen, lysis buffer and elution volumes. Use RNA samples immediately or store at -70 °C.

Extraction kit	Patient specimen	Lysis buffer	Elution volume
QIAamp Viral RNA Mini Kit	140 ㎕	560 ㎕	40 ㎕

6.3 RT-qPCR preparation

① Mixture Preparation
*Mixture preparation should be performed in designated areas to avoid contamination.

i) Prepare mixtures in PCR tubes according to the indicated volumes in the following table.

Mixture components	1 Reaction (Total volume : 15 ㎕)	Volumes for N specimens (㎕)
Master mix	10 ㎕	10 x (N+2)
Primer Probe mix	1 ㎕	1 x (N+2)
DEPC DW	4 ㎕	4 x (N+2)

ii) Pipette 15 ㎕ of each assay mixture into applicable wells.
Cover and transfer the plate into sample processing area.

② Sample Preparation
*Sample should be prepared in area designated for sample preparation.
i) Add 5㎕ of the extracted RNA, control, and NC(DEPC DW) to the wells pre-filled with the assay mixtures.
ii) Seal the plate with sealing film and spin down the plate in a table top plate centrifuge.
iii) Insert the plate into the PCR instrument.

6.4 Software setting

For each PCR instrument and software, enter the following assay settings for the 1copy™ COVID-19 qPCR 4plex Kit.
① Enter the reaction volume as 20 ㎕ and modify PCR conditions as below.

Step	Temperature	Time	Cycle
RT	55 °C	5 min	1
Incubation	95 °C	3 min	1
Amplification	95 °C	5 sec	40
	60 °C *	12 sec	

* Measure fluorescence at 60 °C.
* For ABI 7500, set the amplification time for 60°C as 28 sec.
* Time taken to run each PCR cycle may vary depending on the instrument used.

② Select the type of measurement fluorescence presented in the following table.

Target	CFX96	7500	Quantstudio5	qTOWER3	Rotor Gene Q	LC480
E gene	FAM			FAM	Green	FAM
RdRp gene	VIC			JOE	Yellow	VIC
N gene	Texas Red			Texas Red	Orange	Red610
IPC	Cy5			Cy5	Red	Cy5

7. Interpretation of Results

7.1 Cut off value

For control, IPC and clinical specimens, the cut off value for each applicable target to be considered "detected" (+) is a Ct value ≤40.

Ct value	Result
≤ 40	Detected (+)
> 40 or N/A	Not Detected (-)

※ Set threshold values and baseline

	Threshold						Baseline	
Target	CFX96	7500	Quantstudio5	qTOWER3	Rotor-Gene Q	LC480	Begin	End
FAM Texas Red VIC Cy5	500	50,000	15,000	7	0.05	Abs Quant/2nd Derivative Max	3	15

7.2 Controls interpretation

Control				Negative Control				Interpretation
FAM	VIC	Texas Red	Cy5	FAM	VIC	Texas Red	Cy5	
+				-				Pass
+/-			-	-				Control Failure / System stability failed / Retest
+/-		-	+/-					
+/-	-	+/-						
-	+/-							
+/-				+				

* In the event of a control failure, specimen results should not be reported. Repeat the test run with new controls.
※ Note: Ct ≤40 = Detected (+), Ct>40 = Not Detected (-)

7.3 Patient specimen interpretation

E gene assay (FAM)	RdRp gene assay (VIC)	N gene assay (Texas Red)	IPC (Cy5)	Interpretation
+			+/-	Positive for SARS-CoV-2[a]
+/-	+	-	+/-	Presumptive Positive for SARS-CoV-2[b]
+	-	+/-		
-	+/-	+		
-			+	Negative for SARS-CoV-2
-			-	Invalid Result[c] Repeat extraction and RT-PCR, if result obtained from a repeated test is invalid, collection of new specimen is recommended.

a) If sufficient biological samples (clinical matrix) are not collected and viral load is high, E gene, N gene, and RdRp gene can be positively detected even if IPC is confirmed as negative.
b) A positive result in single, or 2 target results may be suggestive of
1) a sample at concentrations near or below the limit of detection of the test,
2) a mutation in the target region in the oligo binding sites, or
3) infection with some other Sarbecovirus (e.g. SARS-CoV or some other Sarbecovirus previously unknown to infect humans), or
4) carry-over contamination by control or patient samples, or
5) other factors.
c) Invalid result due to potential sampling error or inhibition.

※ Note: Ct ≤40 = Detected (+), Ct>40= Not Detected (-)

CONTACT

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