

1copy™

COVID-19/FluA/FluB/RSV qPCR Kit

Quick Guide

Cat no. M25MD100F

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/FluA/FluB/RSV qPCR Kit is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2, Influenza A virus(FluA), Influenza B virus(FluB) and Respiratory Syncytial Virus (RSV) 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 provider. 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.

2. Kit Contents (Materials Provided)

Kit contents	Cap color	Volume (100 Tests)
Master Mix	Red	1000 µl
Primer/Probe mix 1 (for CFX96™)	Brown (Amber tube)	100 µl
Primer/Probe mix 2 (for Quantastudio5 and 7500)	Brown (Amber tube)	100 µl
Control	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.

※ NOTE

Please note that the Primer/Probe mix tube used is different depending on the real-time PCR instrument
CFX96™ Real-Time PCR Detection System : Primer/Probe mix 1
Applied Biosystems Quantstudio5 and 7500 Real-Time PCR Instrument system : Primer/Probe mix 2

3. 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.

4. 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 5 below)
- QIAamp Viral RNA Mini Kit (Qiagen, Cat no.52904)
- Ethanol (96~100%)

5. Compatible Real-time PCR Instruments

- Applied Biosystems Quantstudio5 (Thermo Fisher Scientific, Product No. A28134, Software version 1.4.3)
- Applied Biosystems 7500 Real-Time PCR Instrument system (Thermo Fisher Scientific, Product No. 4345241, Software version 2.0.6)
- CFX96™ Real-Time PCR Detection system (Bio-Rad, Product No. 1854095-IVD, Software Bio-Rad CFX Maestro version 1.1)

6. Procedure

6.1 Specimen collection, transport and storage

Inadequate specimen collection, improper specimen handling and/or transport 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) <https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html>

Follow specimen collection devices manufacturer instructions for proper collection methods.

After obtaining specimen, place swabs immediately into sterile tubes containing 2-3 ml of viral transport media or universal transport media. The swab specimens 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)

6.3 RT-qPCR preparation

① Mixture Preparation

*Mixture should be prepared in area designated for mixture preparation 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 µℓ)	Volumes for N specimens (µℓ)
Master mix	10 µℓ	10 x (N+2)
Primer Probe mix	1 µℓ	1 x (N+2)
DEPC DW	4 µℓ	4 x (N+2)

ii) Pipette 15µℓ 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.

- Add 5µℓ of the extracted RNA, control, and NC(DEPC DW) to the wells pre-filled with the assay mixtures.
- Seal the plate with sealing film and spin down the plate in a table top plate centrifuge.
- 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/FluA/FluB/RSV qPCR Kit.

① Enter the reaction volume as 20 µℓ 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
	62°C *	12 sec	

- * Measure fluorescence at 62°C
- * For ABI 7500, set the amplification time for 62°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.

Target	Chanel
FluA	FAM
FluB	VIC
SARS-CoV-2	Texas RED
RSV	Cy5
IPC (CFX96)	Quasar705
IPC (ABI7500, ABI Quantstudio5)	TAMRA

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 (-)

Ct values above 40 for FAM, Texas Red, VIC, Cy5, TAMRA and Quasar 705 may be a result of unspecific amplification.

The analytical cut-off value for this product is 40, but this value can be readjusted, depending on the environment of the laboratory.

※ Set threshold values and baseline

Channel	Threshold			Baseline	
	CFX96	ABI 7500	ABI Quantstudio5	Begin	End
FAM, VIC, Texas RED, Cy5	500	50,000	15,000	3	15
Quasar 705	500	-	-	3	15
TAMRA	-	25,000	15,000	3	15

7.2 Controls interpretation

Control					Negative Control					Interpretation
FAM	VIC	Texas Red	Cy5	Q705 or TAMRA	FAM	VIC	Texas Red	Cy5	Q705 or TAMRA	
+					-					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

FluA (FAM)	FluB (VIC)	SARS-CoV-2 (Texas Red)	RSV (Cy5)	IPC (Q705 or TAMRA)	Interpretation
+	-	-	-	+/-	Positive for FluA ^{a)}
-	+	-	-	+/-	Positive for FluB ^{a)}
-	-	+	-	+/-	Positive for SARS-CoV-2 ^{a)}
-	-	-	+	+/-	Positive for RSV ^{a)}
+	+	-	-	+/-	Positive for FluA and FluB ^{a)}
+	-	+	-	+/-	Positive for FluA and SARS-CoV-2 ^{a)}
+	-	-	+	+/-	Positive for FluA and RSV ^{a)}
-	+	+	-	+/-	Positive for FluB and SARS-CoV-2 ^{a)}
-	+	-	+	+/-	Positive for FluB and RSV ^{a)}
-	-	+	+	+/-	Positive for SARS-CoV-2 and RSV ^{a)}
+	+	+	-	+/-	Positive for FluA, FluB and SARS-CoV-2 ^{a)}
+	+	-	+	+/-	Positive for FluA, FluB and RSV ^{a)}
+	-	+	+	+/-	Positive for FluA, SARS-CoV-2 and RSV ^{a)}
-	+	+	+	+/-	Positive for FluB, SARS-CoV-2 and RSV ^{a)}
+	+	+	+	+/-	Positive for FluA, FluB, SARS-CoV-2 and RSV ^{a)}
-	-	-	-	+	Negative for FluA, FluB and RSV
-	-	-	-	-	Invalid Result ^{b)} Repeat extraction and RT-PCR, if repeat result in invalid, consider collection of the new specimen

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

- If sufficient biological samples (clinical matrix) are not collected and viral load is high, SARS-CoV-2, FluA, FluB and RSV can be positively detected even if IPC is confirmed as negative.
- Invalid result due to potential sampling error or inhibition.

CONTACT

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