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Product Information IdPath™series

IdPath™ COVID-19 Real-Time RT-PCR Kit

IP2000	**100 RXN**	
	One-Step RT Enzyme Mix	200 µl
	2X One-Step qPCR Master Mix	1 ml
	Primers/probes Mix	200 µl
	COVID-19 Control	30 µl
	ddWater	1 ml

Storage

Aliquot to avoid multiple freeze-thaw cycles
Protect fluorogenic probes from light

-20°C for 12 months

For Research use only

Description

IdPath™ COVID-19 Real-Time RT-PCR Kit is a real-time RT-PCR test intended for the detection of SARS-CoV-2 virus RNA which might be extracted from the respiratory tract specimens. The Kit provides reagents for multiplex real-time RT-PCR to detect SARS-CoV-2 by one-step reaction, specifically targeting the E (Envelope), RdRP (RNA-dependent RNA polymerase) and N (Nucleocapsid protein) gene for SARS-CoV-2 virus.

The Kit contains the RT enzyme mix and qPCR master mix for reverse transcription and real-time PCR of virus RNA. The COVID-19 Control (positive control) and ddWater (negative control) are used as indicators to avoid false negative/positive results across all experimental procedures. The Primers/probes Mix contains multiplex primers and TaqMan probes specific to the N, E/RdRP genes of SARS-CoV-2 and RNase P gene of human, detected by FAM, VIC and ROX channels, respectively.

Features

- **High Sensitivity**：5×10^2 copies/ml (10 copies/rxn)
- **High Inclusivity**：>99% of currently available complete virus genomes for SARS-CoV-2 including Omicron variant
- **High Accuracy**：Clinical validation with 100% accuracy
- **High Stability**：37/25°C for 2 weeks; 4°C for 24 weeks; 10 times of freeze-thaw cycles
- **High Compatibility**：Suitable for most laboratory qPCR machines
- **Operation Control**：Including internal control for quality control of total process
- **Convenience**：Multiplex (E/RdRP and N) detection by one-step reaction.

Kit contents (Materials provided)

Component	Cap Label (color)	Amount
One-Step RT Enzyme Mix	E (Red)	200 µl
2X One-Step qPCR Master Mix	B (Green)	1 ml
Primers/probes Mix	A (Amber tube)	200 µl
COVID-19 Control	PC (Blue)	30 µl
ddWater*	NC (White)	1 ml

* ddWater is also used as a negative control.

Compatible real-time PCR instruments

- CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad)
- LightCycler® 96 and 480 System (Roche)
- Applied Biosystems StepOnePlus and 7500 Fast Real-Time PCR Instrument System (Thermo)
- Rotor-Gene Q 5plex HRM (Qiagen)

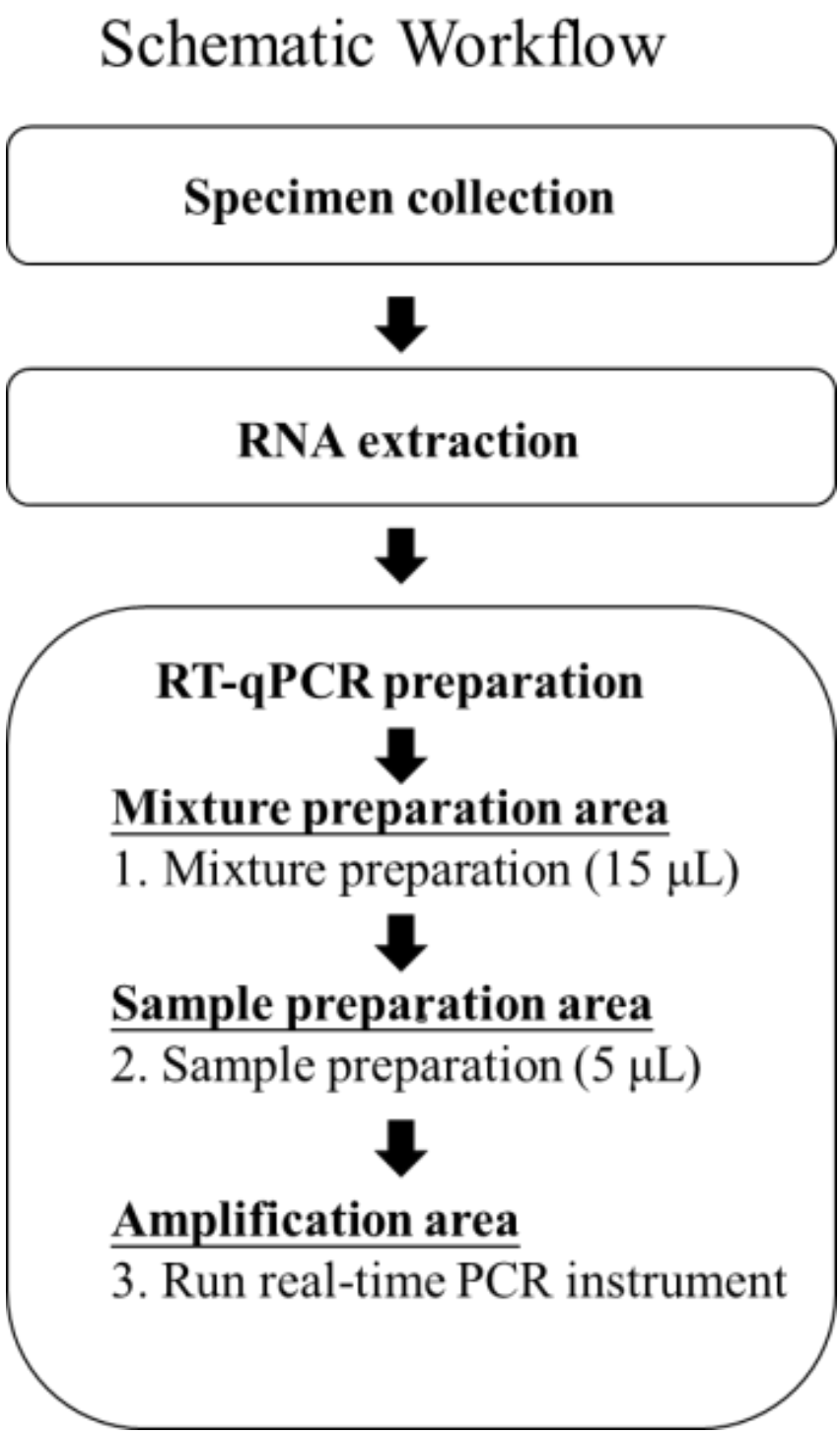
Materials Required but Not Provided

- QIAamp Viral RNA Mini Kit (Qiagen, Cat no. 52904)
- RNase/DNase free disposable latex or vinyl gloves
- Microliter pipettes (1~10 µl, 10~100µl, 100~1000 µl)
- Filter tips
- Vortex mixer
- Benchtop centrifuge (1.5 ml microcentrifuge and 96 well plate centrifuge)
- Ice or cooling/cold block
- 96-well PCR plates and sealing film
- qPCR instrument and manufacturer's instructions

Reagent storage and handling

- Reagents, master mix, and RNA must be thawed and kept on a cold block at all times during preparation and use.
- Do not push air into the assay mixtures and do not mix by violently pipetting up and down to avoid bubble generation.
- Be sure not to introduce any foam or bubbles into the wells of 96-well plates when aliquoting reaction mix and nucleic acid.
- As adding sample and control, immerse the tip below the surface of the liquid. Then press down the push button smoothly to the first stop.

Procedure


Schematic Workflow
Specimen collection
RNA extraction
RT-qPCR preparation
Mixture preparation area
1. Mixture preparation (15 μL)
Sample preparation area
2. Sample preparation (5 μL)
Amplification area
3. Run real-time PCR instrument

Specimen collection, transportation and storage

1. Training in specimen collection is highly recommended due to the importance of specimen quality. For collecting the specimen please refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html.
2. Specimens should be collected into sterile tubes containing viral transport media or universal transport media immediately. Store specimens at 2-8°C and ship overnight on ice pack. If a specimen is frozen at ≤-70°C, ship overnight on dry ice. The specimens for testing can be stored up to 72 hours at 2-8°C. Specimens received frozen should be stored at ≤-70°C until processing.

RNA extraction

Extract the RNA using the QIAamp Viral RNA Mini Kit (Qiagen) according to the manufacturer's instructions.

1. Recommendations: Utilize 140 µl of sample and 560 µl of Buffer AVL with carrier RNA and elute with 50 µl of elution buffer.
2. After the nucleic acid extraction is completed, each eluent should be added into reaction mixture in the well immediately or store at -70°C until processing.

Extraction kit	Patient specimen	Lysis buffer	Elution volume
QIAamp Viral RNA Mini Kit	140 µl	560 µl	50 µl

RT-qPCR preparation

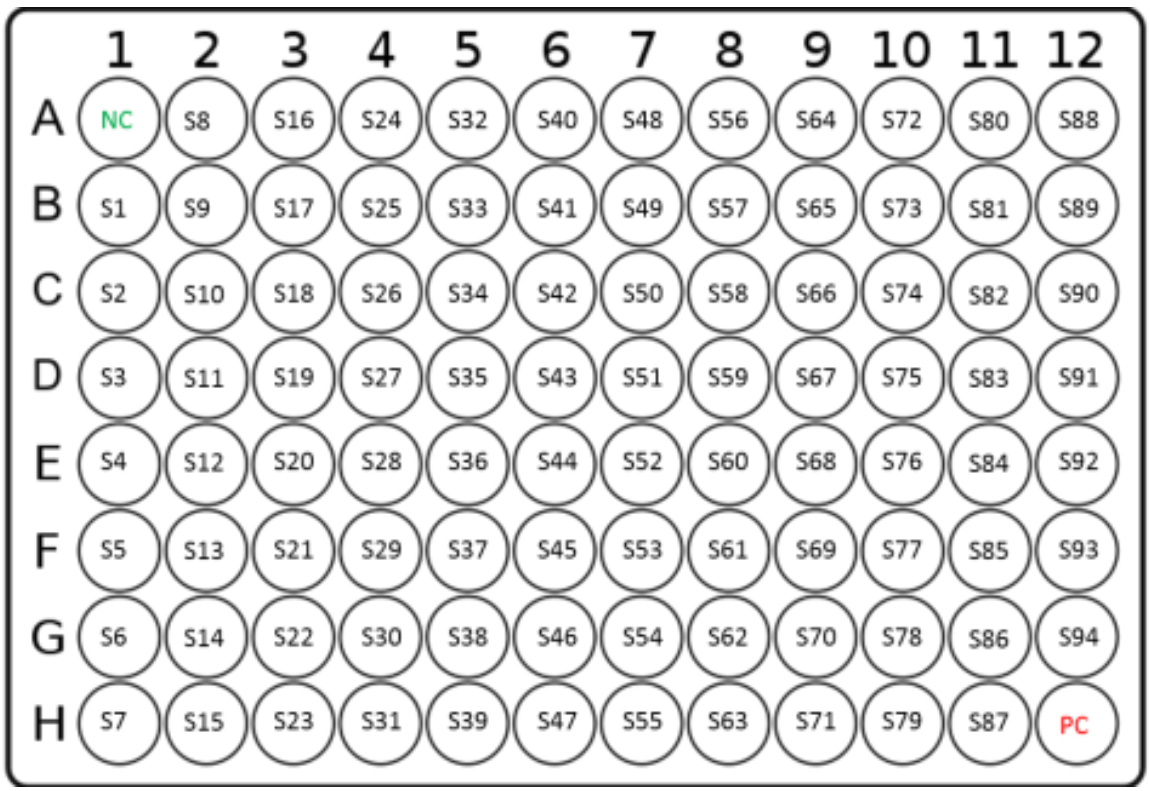
Mixture preparation

* Keep the mixtures on ice or on the cold block and avoid bubble generation during pipetting.

1. Prepare assay mixtures in tubes according to the following table.

Mixture components	1 Reaction (Total 15 µl)	Volumes for N specimens (µl)
Master Mix (B, Green)	10 µl	10 x (N+4)
Enzyme Mix (E, Red)	2 µl	2 x (N+4)
Primers/probes Mix (A, Amber)	2 µl	2 x (N+4)
ddWater	1 µl	1 x (N+4)

2. Pipette 15 µl of each assay mixture into applicable wells according to the plate layout shown below. Cover and transfer the plate into sample processing area.



- NC: Negative control (ddWater)
- PC: Positive control for the E/RdRP, N and RNase P genes
- S: RNA sample

Sample preparation

*Avoid bubble generation when adding samples and keep plate on ice.

1. Add 5 µl of the NC (ddWater), extracted RNA and PC sequentially to the wells of 96-well PCR plate pre-filled with the assay mixtures.
2. Seal the plate with sealing film and spin down the plate in a table top plate centrifuge.
   * Centrifugation at 1000 x g for 1 minute is suggested to eliminate bubbles in the well and to remove moisture on the plate.
3. Make sure no moisture remains, which may cause drift of amplification curve.
4. Run on the qPCR machine immediately after sample preparation is completed.

Software setting

For each PCR instrument and software, enter the assay settings as follows.

1. Enter 20 µl for the reaction volume and modify PCR reaction conditions as presented in the following table.

Step	Temperature	Time	Cycle
RT	50℃	10 min	1
Incubation	95℃	1 min	1
Amplification	95℃	3 sec	40
	58℃	30 sec	

2. For CFX96 (Bio-Rad), select the type of fluorescence and set threshold values and baseline as presented in the following table.

CFX96 (Bio-Rad)			
Channel	Threshold	Baseline	
		Begin	End
FAM	120	6	15
VIC	200		
ROX	200		

3. For the other PCR instruments and software, please refer to Software Setting guide (scan QR code at the section of Support/Documents).

Interpretation of results

Cut off value

Ct value	Result
< 37	Detected (+)
≥ 37 or Undetermined	Not Detected (-)

Interpretation

E/RdRP Result (VIC)	N Result (FAM)	RP Result (ROX)	Interpretation
+	+	+/-	Positive for COVID-19
+	-	+/-	Positive for COVID-19*
-	+	+/-	Positive for COVID-19*
-	-	+	Negative for COVID-19
-	-	-	Invalid

* Consider repeat of RT-PCR for further confirm.

Limitation

- This product is only used for research purpose.
- False positive and false negative results can be caused by poor specimen quality, improper specimen collection, improper transportation, improper laboratory processing, or a limitation of the testing technology.

Support/Documents

Software setting guide

Supplement

Troubleshooting

The latest version of the manual can be downloaded from www.smobio.com/shop.