Intended Use

This SARS-CoV-2 Real-time RT-PCR Detection Kit **(Lyophilized)** is used for the detection of Coronavirus RNA expression in COVID-19 suspect patient's specimens. The detection result is only for clinical reference, and it cannot be used as the sole basis for clinical diagnosis and treatment.

For in vitro diagnostic use only.

Suitable specimen types

Upper respiratory specimen (including nasal swabs, nasopharyngeal swabs / aspirates / washes, and sputum).

Lower respiratory specimen (including respiratory aspirates, bronchial washes, bronchoalveolar lavage fluids, and lung biopsy specimen).

Principle

The SARS-CoV-2 Real-time RT-PCR Detection Kit is a triple real-time fluorescent RT-PCR assay. All components are premixed and lyophilized, providing more stable detection performance.

The conserved sequences in SARS-CoV-2 ORF1ab gene and N gene are selected as amplification target regions. The probe designed for ORF1ab gene is labeled with FAM, and the probe designed for N gene is labeled with HEX. The human Rnase P gene is labeled with Cy5 as an internal control, which monitors RNA isolation from specimen collection, or indicates if there is non-specific interference.

Kit Contents(100T)

SARS-CoV-2 Real-time RT-PCR Detection Kit includes components as below:

Component	Package	Quantity
SARS-CoV-2 RT-PCR Mix (Lyophilized)	Vial	2 Vials×50T
Positive Control (Lyophilized)	Tube	1×100 μL
Resuspension Buffer RB	Tube	2×1mL
PCR grade Water	Tube	1×1mL
Negative Control	Tube	1×1mL

Note: Do not mix the components from different batches for detection.

Transport & Storage Conditions

Kit	Transport condition	Storage Condition	Shelf life
SARS-CoV-2 Real-time RT-PCR Detection Kit (Lyophilized)	Ambient Temperature	20℃~-15℃	24 months

Note: Ambient temperature transportation shall not exceed three months.

Other required materials and devices

1. Real-time PCR instrument with FAM, HEX and Cy5 channels.
2. Reagents and Devices for viral RNA isolation.
3. Centrifuge for 1.5 mL tubes and PCR-well strips or 96-well plate (if available).
4. Nuclease-Free pipette tips with filters.
5. Nuclease-Free microcentrifuge tubes.
6. Nuclease-Free PCR tubes or plates and seals that are compatible with your specific qPCR platform.
7. Disposable gloves.

Preparation of RT-PCR Reaction Mixes

1. Reagent Preparation

a. Resuspend the Lyophilized SARS-CoV-2 RT-PCR Mix with 800μL Resuspension Buffer RB per vial, and mix gently. The RT-PCR mix is ready for use. If it is not immediately used, the mix must be stored at -20℃ after resuspending. (Note: Avoid of the freeze-thawing over three times).

b. Resuspend Positive Control: resuspend with 100μL PCR grade Water. (Once the positive control has been resuspended, store it at -20ºC. Use it up in 7 days.).

2. Set up RT-PCR reaction

a. Add 15μL of the resuspended RT-PCR mix for each PCR reaction. The reaction tube should be freshly prepared just before use.

b. Add 5μL of RNA sample, or Positive Control / Negative control into different wells according to your plate setup.

c. Sealing the PCR tubes or plate tightly with the caps or film.

d. Shortly spin the tubes or plate to remove bubbles.

3. Set up PCR Program

Put the tubes in your qPCR instruments. Setup the running program as below.

Step	Cycles	Temperature	Time	Fluorescence
Reverse transcription	1	50ºC	10 min	—
Initial denaturation	1	95℃	2 min	—
qPCR amplification	45	95ºC	5s	—
		60℃	20s	FAM, HEX, Cy5

Result interpretation

Make sure the following two prerequisites are fulfilled:
1. Negative control: No amplifications in FAM, HEX and Cy5 channels.
2. Positive control: Cq < 35 in FAM and HEX channels

Please interpret the results with the following metric:

ORF1ab gene （FAM）	N gene （HEX）	Internal Control （Cy5）	Interpretation of Results
−	−	+	SARS-CoV-2 Negative
+	+	+	SARS-CoV-2 Positive
−	+	+	SARS-CoV-2 Positive
+	−	+	SARS-CoV-2 Positive
+	+	−*	SARS-CoV-2 Positive
−	+	−*	SARS-CoV-2 Positive
+	−	−*	SARS-CoV-2 Positive
−	−	−*	SARS-CoV-2 Negative

＋: typical S-shape amplification curve and Cq≤40.
—: no typical S-shape amplification curve and no Cq value or Cq>40.
If typical S-shape amplification curve showed and Cq>40 in FAM or HEX channels, repeated test is needed.

**The Cy5 negative result (—) indicates there is no human Rnase P gene or too few to detect. Except the negative control or non-human specimens, the result indicates that there is a problem with the experiment (failure specimens collection, failure RNA isolation, interfering substances,etc.). We recommend looking into the matter and take action.*

Performance Index

Specificity: no cross-reaction with other respiratory pathogens such as HCoV-NL63, HCoV-HKUI, HCoV-229E, Influenza A viruses, HCoV-OC43, Influenza B virus, SARS coronavirus, MERS coronavirus, Canine coronavirus, Influenza A virus subtype H7N9, and human genome DNA.
Accuracy: positive coincidence rate and negative coincidence rate of enterprise reference is 100%.
Minimum LOD: 100-150 copies/ml sample.
Clinical evaluation: The diagnostic sensitivity and diagnostic specificity were 98.04 % and 100.00%.

General Precautions

- Ensure that adequate standard operating procedures (SOPs) are in use.
- The kit is intended for use by laboratory trained personnel. Ensure that operators are trained for appropriate specimen collection, storage, packaging, and transport.
- Ensure rational guidelines and recommendations on laboratory safety are followed in all circumstances.
- Do not use the kit beyond its shelf life.
- All specimens collected for laboratory investigation should be regarded as potentially infectious.
- Specimens must be collected, transported, and stored using the exact procedures and conditions. Improper collection, transport, or storage of specimens may impact the performance of the test.
- There is a possibility of false positive results due to cross-contamination, either samples containing high concentrations of target RNA or contamination due to PCR products from previous reactions.
- Wear appropriate personal protective clothing, including gowns, disposable gloves and eye protection, throughout the assay procedure.
- Specimen processing should be carried out in a certified BSL-2 laboratory following biosafety level 2 guidelines or higher.
- Gloves must be changed and disposed before leaving the area.
- Thoroughly clean and disinfect, periodically, all work surface and devices.
- To avoid cross-contamination, workflow in the laboratory must be in uni-directional manner from sample preparation area to Pre-Amplification Area to PCR-Amplification Area.
- All materials used in the area should be stayed at that area and should not be moved or used in other areas. After the assay procedures, the workbench and lab supplies should be cleaned and disinfected immediately.
- Use isolated RNA to test immediately after extraction or store at -20ºC (short time)/-70ºC.
- Before disposal, all waste materials should be autoclaved or incinerated. Dispose of all waste materials according to National Legislation.

Symbols meaning

CE	CE Symbol	IVD	In vitro diagnostic medical device
	Keep away from sunlight		Keep dry
	No secondary use		Reference instructions
REF	Reference Number	LOT	Lot Number
	Use By	Σ	Number of Tests
	Temperature Limitation		Damaged packing, do not use
	Manufacturer Name Address	EC REP	Name and Address of EuropeanUnionRepresentative

Manufacturer Information

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