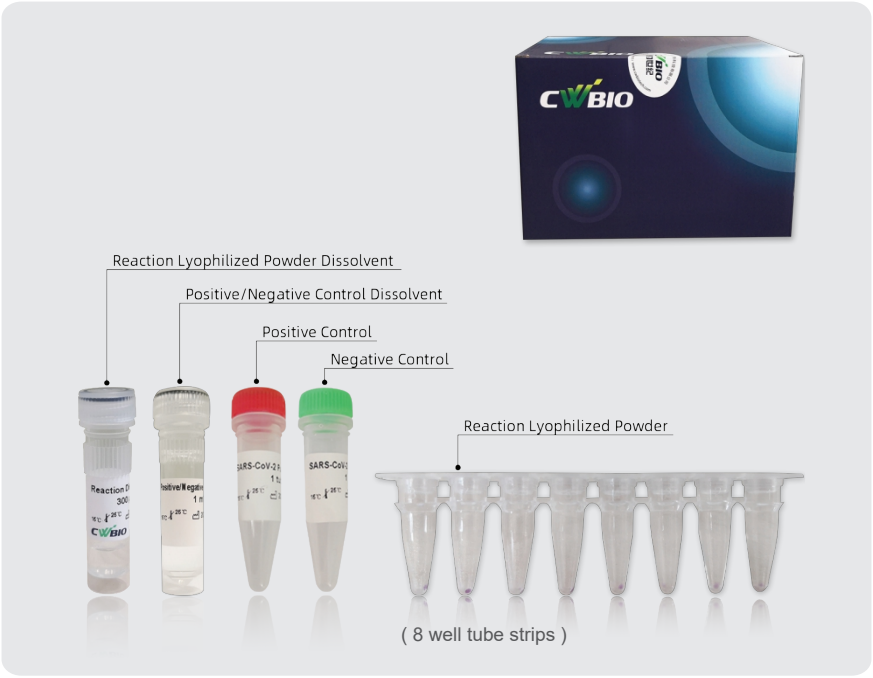
Operation Manual-Extraction

Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing)

Components



Size

48 tests/Box, 96 tests/Box

Kit Components

Reagent	Component	Size (48 tests/Box)	Size (96 tests/Box)
Reaction Lyophilized Powder	Reverse transcriptase, hot start DNA polymerase; specific ORF1ab and N gene primers, probes; internal control primers, and probes; reaction buffer, etc. (Lyophilized powder)	8 well tube strip×6	8 well tube strip×12
Reaction Lyophilized Powder Dissolvent	Divalent cation, buffer and monovalent cation, etc	300 μL/tube ×1	300 μL/tube ×2
SARS-CoV-2 Positive Control	Pseudovirus carrying target gene and plasmids carrying internal control (Lyophilized powder)	tube×1	tube×2
SARS-CoV-2 Negative Control	Plasmid not carrying target genes（Lyophilized power）	tube×1	tube×2
Positive/ Negative Control Dissolvent	Tris, EDTA, NaOH, etc.	1ml/tube×1	1ml/tube×2

Storage Condition

The kit can be stored and transported at room temperature (10-30°C) for a long time before use. After the reagent box is opened for use, it can be stored at room temperature within one month. If it is stored for a long time, it is recommended to place the reagent box at 2-8°C.

The kit production date and expiration date are shown in the outer packaging box, and the validity period is 12 months.

Operating steps

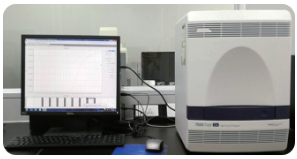
Everything you need to detect for COVID-19 in the testing process

Vortex mixer

Mini centrifuge

Pipette

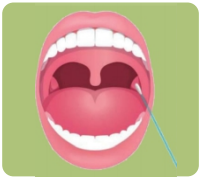
Tips with filter



Real-time fluorescence quantitative PCR instrument

Sample collection and processing

The CWBIO or other manufacturers virus preservation solution is used to preserve swab samples.



1. Collect sample from the subject

2. Put the swab into the collection tube and break off the swab head

3. Lighten the cover. Shake it up and down several times for mix throughly. Then the sample is ready to use for PCR.

Nucleic acid extraction

Extract nucleic acids from samples using a nucleic acid extractor, -20°C storage.

Reaction Lyophilized Powder dissolving — Reagent preparation area:

- Take out the Reaction Lyophilized Powder Dissolvent and Reaction Lyophilized Powder（8 well tube strip）at reagent preparation area, then centrifuge for 10s.
- Take N PCR reaction tubes (N= n + 2 (Positive Control +Negative Control) adding 5 μL Reaction Lyophilized Powder Dissolvent into each tube of Reaction Lyophilized Powder.
- Cover the lid of PCR reaction tubes tightly. Vortex the prepared PCR reaction tubes for 15s, then centrifuge for 10s.
- Transfer them to the sample processing area.

Sample processing — Sample processing area

- Take out the Negative Control Lyophilized Powder、Positive Control Lyophilized Powder and Positive/Negative Control Dissolvent at reagent preparation area, then centrifuge for 10s.
- **SARS-CoV-2 Positive Control dissolving:** add 500 μL Positive/Negative Control Dissolvent into SARS-CoV-2 Positive Control lyophilized powder. Lighten the cover.
- **SARS-CoV-2 Negative Control dissolving:** add 500 μL Positive/Negative Control Dissolvent into SARS-CoV-2 Negative Control lyophilized powder. Lighten the cover.
- Vortex the Positive/Negative Control tubes for 30s, then centrifuge for 10s.
- Adding nucleic acids samples and controls to reaction reagent: add 20 μL samples respectively in sequence, add 20 μL Negative Control to one of PCR reaction tubes and add 20 μL Positive Control to one of PCR reaction tubes.
- Cover the lid of PCR reaction tubes tightly. Vortex the prepared PCR reaction tubes for 30s, then centrifuge for 10s.

PCR Amplification — PCR amplification area

▪ Open the 7500. Put 8-tube strips into the fluorescence quantitative PCR instrument and start the instrument.

▪ Open the parameter window to set the cycle conditions, and the reaction procedure is shown in Table 1.

Table 1 Reaction Procedure

Procedure	Temperature	Time	Cycle
Reverse transcription	55°C	1 min	1
Pre-denaturation	96°C	20 sec	1
Denaturation	96°C	5 sec	45
Annealing, extension and Collect fluorescence signal	58°C	30 sec	

▪ Setting the qPCR instrument parameters following the instructions of the manual. Here is an example of ABI7500 setting.

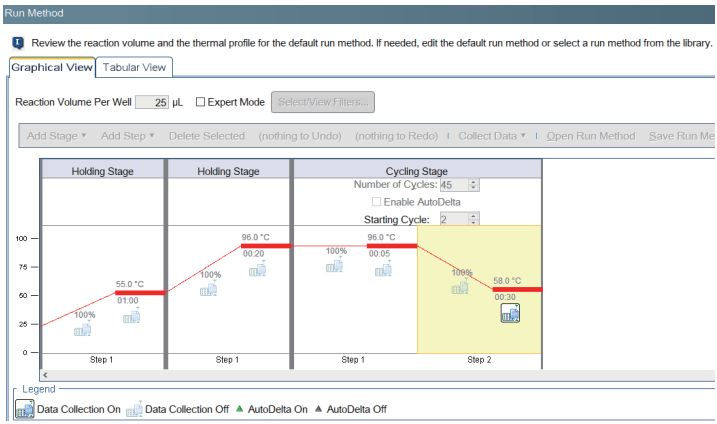


Figure 1 ABI7500 parameters setting

Note: After the amplification completion, please removed PCR reaction tubes from the instrument and drop into self-sealing bags. (Please note: before drop tubes , check the tightness of tube lid of 8 well tube strips, otherwise, any lid loose would cause aerosol pollution)

Interpretation of Test Results

Note: FAM is for ORF1ab gene, ROX is for N gene, and VIC is for internal control gene.

Table 2 Positive control and negative control

CT Value			Control Interpretation
IC (VIC)	ORF1ab (FAM)	N (ROX)	
CT≤35	CT≤35	CT≤35	Positive control
CT≤35	CT>40 or None	CT>40 or None	Negative control

The positive control and negative control are consistent with the above table, which proves that the operation is OK and the kit are effective. After that, real sample interpretation can be carried out.

CT Value			Control Interpretation
IC（VIC）	ORF1ab （ FAM）	N（ROX）	
/	CT≤35	CT≤35	Positive
/	CT≤35	CT>40 or None	Positive
/	CT>40 or None	CT≤35	Positive
/	CT≤35	35<CT≤40	Positive
/	35<CT≤40	CT≤35	Positive
/	CT>40 or None	CT>40 or None	Negative
/	35<CT≤40	CT>40 or None	Retest*
/	CT>40 or None	35<CT≤40	Retest*
/	35<CT≤40	35<CT≤40	Retest*
CT>40 or None	CT>40 or None	CT>40 or None	Invalid sample (Resampling)

/ indicate follow situations：CT≤35 or 35<CT≤40 or CT>40 or None.

*Retest result that is not negative as listed in the table is positive.

Amplification curve with CT value is typical S-type with obvious exponential growth period. If you encounter some special results that are difficult to interpret, our technicians will guide you to interpret.