Hymon™ SARS-CoV-2 Test Kit Instruction for Use

Package Size / Cat.No.

96 tests / cat.no. 351251

Intended Use

The kit is made available for *in vitro* detection of viral RNA of SARS-CoV-2.

The kit applies real-time RT-PCR method for laboratory detection of viral RNA of SARS-CoV-2 in nasopharyngeal/oropharyngeal swab samples. The test results may serve as references to clinical evaluation of COVID-19.

Technical Principles

Based on high-efficiency enzymatic processing technology, sample processing and impurities removal were achieved in one single tube along with releasing and stabilization of nucleic acids including viral RNA at the same time. The RT-PCR test takes N and E gene sequences of SARS-COV-2 as PCR target regions. Human genomic sequence target is made use as internal quality control.

Reagents

Item	Size	Amount	Contents
Sample reagents			
LY Buffer	500 μL	1 tube	Tris
PN Buffer	50 μL	1 tube	Lysozyme
Nucleic acid detection reagents			
PM Buffer	1.3 mL	1 tube	Primers & probes, dNTPs, $MgCl_2$
RE Mix	100 μL	1 tube	Reverse transcriptase, Taq DNA polymerase
Controls			
PC	25 μL	1 tube	Synthetic RNA
NC	900 μL	1 tube	H_2O

Note:

NC should participate in sample processing as a sample.

PC does not participate in sample processing and should be directly added into the PCR mix (see Test Procedures section).

Storage and Stability

Hymon™ SARS-CoV-2 Test Kit should be stored at -20℃ and the reagents are valid for 12 months.

Note: the specific production and expiry date of the product can be found on the kit label. Do not uses beyond the expiry date.

Instrument

Applied Biosystems 7500 Real-Time PCR System.

Sample Processing

1. Nasopharyngeal/oropharyngeal swabs should be collected according to standard clinical practice procedures and stored in Phosphate Buffer Saline (PBS).

2. Freshly collected samples can be used for test immediately or stored at -20±5℃ for no more than 3 months. Please avoid repeated freezing and thawing more than three times.

Test Procedures

1. Sample processing (sample preparation area)

1.1 Refer to the table below to prepare LY-PN working solution (LY-PN) according to the actual quantity:

Reagent	LY Buffer	PN Buffer
Volume	4.5 μL	0.5 μL

1.2 Flush Flush spin after the contents are fully mixed to settle down to the bottom of the tube. Add 45 μL swab elute/NC to a 1.5 mL tube, and then add 5 μL LY-PN in each tube. Vortex the tube to mix the liquid and then flush spin the contents to the bottom of the tube.

1.3 Incubate the microtubes containing the samples at 58 °C for 10 min, 95 ℃for 2 min.

1.4 The product is ready for directly use in PCR reactions.

Note: the negative control in this kit participates in sample processing and is used to monitor the testing process; the positive control does not participate in the extraction and is used for the quality control of PCR detection reagents.

2. PCR reagent preparation (at reagent preparation area)

2.1 Take the PM Buffer out of the kit, melt at room temperature, vortex and mix well, flush spin to settle done contents to the bottom of tube.

2.2 Refer to the table below to prepare PCR mix according to the actual quantity:

Reagent	PM Buffer	RE Mix
Volume	13 μL	1 μL

2.3 Flush spin after the contents are fully mixed to settle down to the bottom of the tube. Add 14 μL prepared PCR mix into each PCR tubes (or wells of PCR microplate).

3. Sample loading (sample preparation area)

Add each 6 μL of the processed negative control, the nucleic acid of the sample to be tested and the positive control into the PCR reaction tubes (or wells of PCR microplate) successively, close the tube lid tight (or seal the microplate), mix thoroughly, and transfer PCR reaction tubes (or microplate) to the amplification detection area after flush spin.

4. PCR amplification (amplification detection area)

4.1 Put the reaction tubes (or microplate) into the sample tank of the instrument.

4.2 ABI instrument settings (take ABI 7500 as an example)

4.2.1 Open the "setup" window, set Negative control (NC), Positive control (PC), Unknown samples (Unknown) according to the corresponding order of samples, and set the sample names in the "Sample Name" column.

4.2.2 Open the "instrument" window and set the cycle conditions as follows:
42℃, 5 min;
94℃, 1 min;
95℃ 15 sec, 60℃ 31 sec, 72℃ 30 sec; the fluorescence signal is collected at 60 ℃, and the signal collection channels are FAM and VIC, 40 cycles.

After setting, save the file and run the program.

5. Result analysis

Automatically save the result after the reaction, and adjust the start value, end value and threshold value of baseline according to the image after analysis. Click "analysis" to obtain the analysis results automatically, and read the test results in the "report" window.

Quality Control

NC: there is no obvious amplification curve of FAM and VIC;

PC: there is obvious amplification curve of FAM and VIC, Ct value ≤ 30;

The above requirements shall be met simultaneously in the same experiment; otherwise, the experiments are invalid and need to be repeated.

Result Interpretation

Detection results		Result	Result interpretation
FAM Ct value	VIC Ct value		
No result or Ct > 38	Ct ≤ 38	SARS-CoV-2 negative	It indicates that there is no RNA of SARS-CoV-2 in the tested sample or its concentration is lower than the limit of detection with the kit
No result or Ct > 38	Ct > 38	Need reexamination	The result is not valid. The cause shall be found out and eliminated, and the sample shall be rechecked.
Ct ≤ 38	Ct ≤ 38 or Ct > 38	SARS-CoV-2 positive	Indicates that the tested sample contains RNA of SARS-CoV-2

Limitations of Tests

The test results of samples are related to the collection, transportation, treatment and preservation of samples. If any of the above links are improperly operated, it may lead to false negative or false positive results of the test.

Product Performance Index

Analytical sensitivity: 5 copies/reaction.

There is no cross reaction with other pathogens such as influenza A virus, influenza B virus, parainfluenza virus, respiratory syncytial virus, adenovirus, dengue virus, SARS, MERS-CoV, OC43, NL63, 229E and HKU1.

Precautions

1. All samples shall be considered as potentially infectious and shall be operated and handled in strict accordance with the laboratory's bio-safety requirements. The experimental personnel should receive professional training (Including sample processing, reagent preparation, instrument operation and software setting, etc.). For the laboratory management specifications, please strictly follow the relevant management specifications for gene amplification test laboratory issued by local regulatory agencies.

2. This kit is only used for in vitro detection.

3. The laboratory should be separated by reagent preparation area, sample preparation area and amplification detection area. Work flow: all the articles in each area are for special purpose, and they shall not be used in a cross way to avoid contamination. Laboratory clothes, hats, shoes, gloves, etc. shall be fully equipped during operation to avoid direct contact of reagents or samples with skin. In case of liquid leakage, wash with plenty of water immediately. In case of contact with skin wound, inform local health and epidemic prevention department in time.

4. The real-time fluorescent quantitative PCR analyzer should be calibrated regularly.

5. Please put the used pipette tips into the waste tank directly, and discard them in a designated manner at a designated place together with other discarded articles according to local regulations.

6. Clean the working area immediately after the experiment. The bench and all kinds of experimental articles should be disinfected with 1% sodium hypochlorite, 75% alcohol or UV light regularly.

Basic Information

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