SARS-CoV-2 Nucleic Acid Detection Kit (Multiplex Real Time RT-PCR)

Revision: A/1

【Product Name】

SARS-CoV-2 Nucleic Acid Detection Kit (Multiplex Real Time RT-PCR)

【Cat. No.】

DV101

【Packaging Specifications】

48 tests/kit 200 tests/kit

【Intended Use】

This kit is intended for *in vitro* qualitative detection of the *ORF1ab* and *N* genes from SARS-CoV-2 in pharyngeal swab or bronchoalveolar lavage specimens collected from Coronavirus Disease 2019 (COVID-19) suspected cases, suspected clusters of cases, or other individuals who need SARS-CoV-2 infection diagnosis or differentiation diagnosis.

The definitions of COVID-19 related groups such as “suspected cases” or “suspected clusters of cases” should be referred to *Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia, Surveillance Protocol for Novel Coronavirus Pneumonia* or other COVID-19 related documents (the latest version) from China CDC.

This kit is only for use in auxiliary diagnosis or storage for emergency use of COVID-19 *in vitro* diagnosis during COVID-19 outbreak since December of 2019. It cannot be used as a conventional *in vitro* diagnosis reagent for clinical practice. The use of this kit should be under the requirements of *Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia, Protocol for Prevention and Control of COVID-19* and other COVID-19 related documents (the latest version).

The nucleic acid detection of SARS-CoV-2 should conform to requirements of COVID-19 related documents such as *Laboratory testing for COVID-19* (the latest version) from China CDC. The biosafety requirements should be strictly complied with.

The detection results of this kit should be regarded as a reference for clinical practice, but not as the sole standard for clinical diagnosis. It is suggested to make a comprehensive analysis combined with clinical symptoms and other laboratory testing methods.

The laboratory personnel for SARS-CoV-2 detection should be professionally trained with gene amplification or molecular biology detection and qualified for related experimental operations. Biosafety protective equipment and programs are required for the laboratories.

【Principles】

The kit is designed for detecting SARS-CoV-2 RNA in specimens using multiplex real time RT-PCR technology with primers and probes targeting the conserved regions of *ORF1ab* and *N* genes . Simultaneously, this kit contains an endogenous control (The internal control *RNase P* gene is detected by Cy5 channel.) to monitor the process of specimen collection, nucleic acid extraction and PCR and reduce false negative results.

【Kit Contents】

Component Name	Main Constituents	Specifications and Quantity (48 tests)	Specifications and Quantity (200 tests)
SARS-CoV-2 PCR Reaction Mix	Reaction buffer, dNTPs, *etc*.	720 µl×1 tube	1000 µl×3 tubes
SARS-CoV-2 PCR Enzyme Mix	Reverse transcriptase, RNase inhibitor, Taq DNA polymerase, uracil-DNA glycosylase (UDG)	48 µl×1 tube	200 µl×1 tube
SARS-CoV-2 PCR Primer/Probe Mix	Primers and probes for *ORF1ab*, *N* genes and the internal control-*RNase P* gene (*RP*)	192 µl×1 tube	800 µl×1 tube
SARS-CoV-2 Positive Control	*In vitro* transcribed RNA for *ORF1ab*, *N* genes and the internal control-*RP* gene	50 µl×1 tube	200 µl×1 tube
SARS-CoV-2 Negative Control	RNase-free Water	50 µl×1 tube	200 µl×1 tube

Note: Components from different lots should not be mixed for use.

【Storage Conditions and Shelf Life】

- Store the kit at -20±5°C away from light for 12 months.
- Ship the kit at low temperature. Dry ice should be used for long-distance shipping. Avoid repeated freeze-thaw cycles (freeze-thaw cycles should be fewer than 10).
- Manufacture date and expiration date are shown on the label.

【Instrument】

Validated Instrument in-house: ABI 7500 Real-Time PCR instrument

Instruments used by customers with revised interpretation for test results : ABI QuantStudio 3, Bio-Rad CFX96.

Please contact our Technical Support team for other instruments.

【Specimen Requirements】

1. Acceptable specimen types: Pharyngeal swab or bronchoalveolar lavage specimens.
2. Sampling of specimen: Follow the specimen sampling method or Laboratory testing for COVID-19 (the latest version) from China CDC.
3. Specimen storage and shipping: Specimens to be used immediately or within 24 hours should be stored at 4°C. Specimens which cannot be used within 24 hours should be stored at or below -70°C. If -70°C is not possible, the specimens to be tested can be stored at -20°C for 10 days and nucleic acid can be stored at -20°C ±5°C for 15 days. Repeated freeze-thaw cycles should be avoided. Specimens should be shipped on ice in sealed foam boxes for transportation or with adding ice constantly on the way.

【Test Method】

1. Specimen Preparation (Specimen Preparation Area)
   Pipet 200 µl of specimen for nucleic acid extraction. Extracted RNA can be used directly for detection. If the extracted RNA is not for the subsequent detection after extraction immediately, it can be stored at -70°C. Avoid repeated freeze-thaw cycles.
2. Reagent Preparation: (Reagent Preparation Area)
   Thaw SARS-CoV-2 PCR Reaction Mix and SARS-CoV-2 PCR Primer/Probe Mix at room temperature. Mix thoroughly to ensure homogeneity, and then centrifuge briefly. Briefly spin down SARS-CoV-2 PCR Enzyme Mix, and put on ice for the next step. Prepare the reaction mix for the number of reactions based on the table below. It is recommended to set up a negative and a positive control for each test. When the number of specimens is **n**, the number of reactions N= the number of specimens (**n**) + positive control (1) + negative control (1) + 1.

Mastermix Preparation Table

Kit Components	Volume per Reaction (μl)
SARS-CoV-2 PCR Reaction Mix	15 μl×N
SARS-CoV-2 PCR Primer/Probe Mix	4 μl×N
SARS-CoV-2 PCR Enzyme Mix	1 μl×N

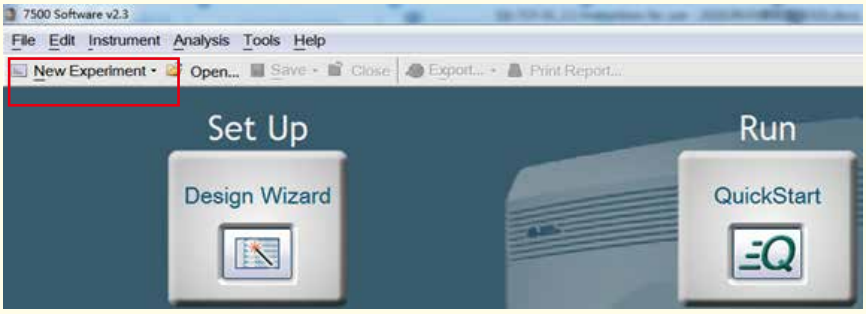
Mix the reagents thoroughly, then dispense equal 20 μl into each microcentrifuge tube, and transfer to the Specimen Handling Area.

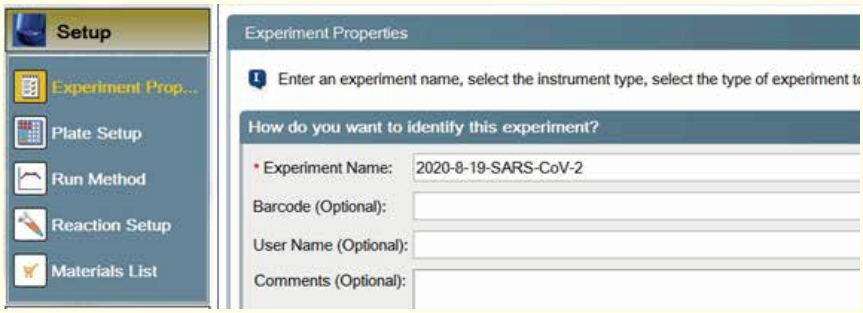
3. Specimen Addition (Specimen Handling Area)

Add 5 μl of extracted specimen nucleic acid, SARS-CoV-2 Positive Control, and SARS-CoV-2 Negative Control to the aliquoted system, to reach a total reaction volume of 25 μl. Tightly cap the reaction tube, then centrifuge briefly at low speed, and move to the Amplification and Analysis Area.

4. PCR Amplification (Amplification and Analysis Area)

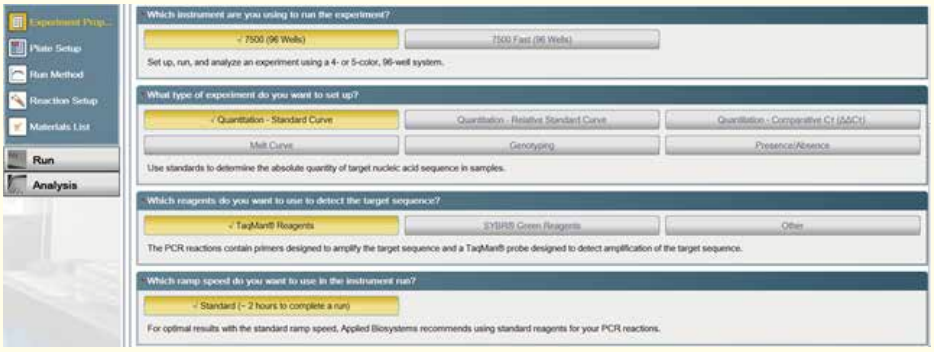
Place the PCR tube in sequence into the PCR instrument. After starting 7500 Software v2.0.5, click **New Experiment** to open the **Setup** menu. In the **Experiment Properties** screen, enter an experiment name such as "2020-8-19-SARS-Cov-2" to identify your experiment.



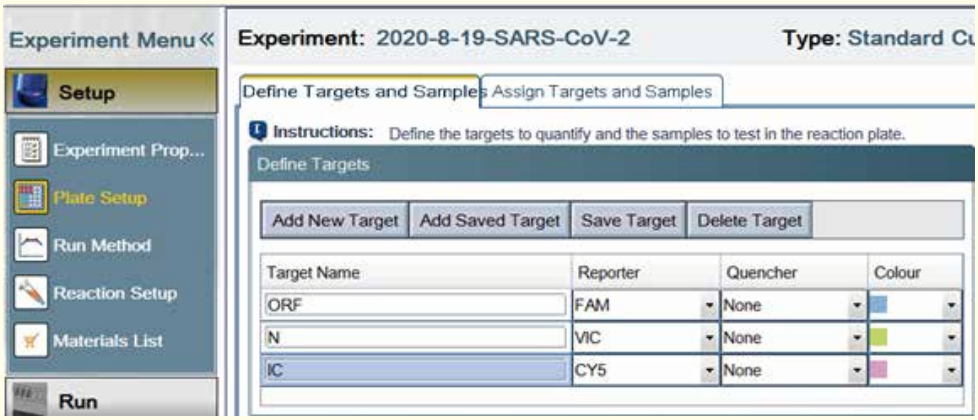


Select **7500 (96 Wells)** for the instrument type, and select **Quantitation-Standard Curve** for the experiment type.

Select **TaqMan Reagents** for the reagents, and select **Standard (~2 hours to complete a run)** for the ramp speed.

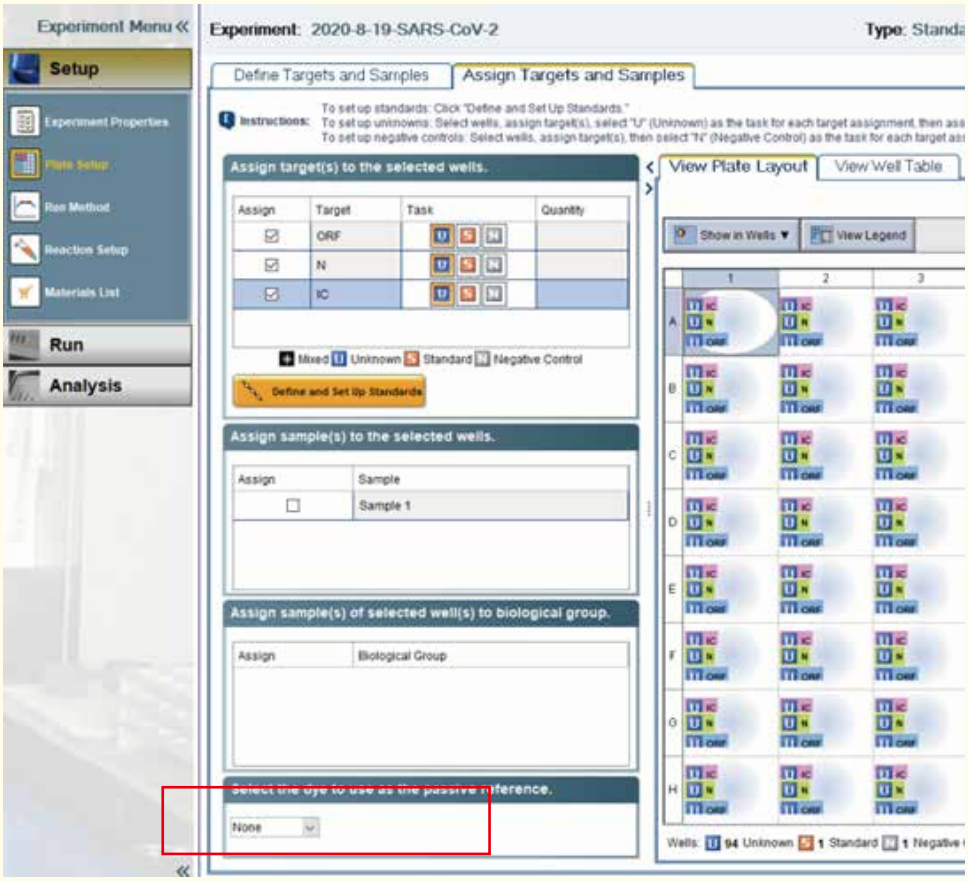


Click “Plate Setup” icon. In the **Define Targets and Samples** screen, click the **Target Name** cell, then enter **ORF**.
In the Reporter drop-down list, select **FAM**. In the Quencher drop-down list, select **None**. In the Color field, leave the default.
In the second row, click the **Target Name** cell, then enter **N**. In the Reporter drop-down list, select **VIC**. In the Quencher drop-down list, select **None**. In the Color field, leave the default.
In the third row, click the **Target Name** cell, then enter **IC**. In the Reporter drop-down list, select **CY5**. In the Quencher drop-down list, select **None**. In the Color field, leave the default.



Click **Assign Targets and Sample** tab. Follow the Instructions in the window to set up standards, unknowns and negative controls.

Select **None** for passive reference.
Click **Run Method**. In the **Run Method** screen, select either the **Graphical View** tab (default) or the **Tabular View**. Click the **Reaction Volume Per Well** field, then enter 25 µl. Configure PCR protocol as shown in the table below. Review the thermal profile.



STEPS	TEMPERATURE	REACTION TIME	CYCLES
Reverse Transcription	50°C	5 min	1
Pre-denaturation	95°C	30 sec	1
Denaturation	95°C	5 sec	45
PCR cycling	60°C	34 sec*	

* means **Data Collection On**.

5. Result Analysis

The results are automatically saved after the reaction. Then analyze the amplification curves of the target genes and internal control gene separately. According to the analysis of the image, adjust Baseline's Start value, End value and Threshold value. Click **Analyze** for analysis, and then record the qualitative results under the Plate window. (As for ABI 7500, the user can adjust manually according to the actual conditions to ensure that all the baselines for the curves are flat. For instance, the Start value can be set from 3 to 15, and End value can be set at 5 to 20. Threshold value should be set right above the summit of the amplification curve of negative control.)

6. Quality Control (Evaluation of Experiment Effectiveness)

Each control in the kit should meet the following requirements, otherwise the experiment is invalid.

	Positive Control	Negative Control
FAM channel（*ORF1ab* gene）	Ct ≤ 32	No Ct value or Ct>40
VIC channel（*N* gene）	Ct ≤ 32	No Ct value or Ct>40
Cy5 channel (internal control gene)	Typical sigmoidal curve，and Ct ≤ 32	No Ct value or Ct>40

【Precautions】

1. Please read the manual carefully before test and follow the protocol strictly.
2. Set both positive and negative controls for each test.
3. Test analysts should be trained by professionals and must perform operation in labs following safety guidelines and wear personal protective equipment.
4. The kits should avoid light for storage to protect the fluorophore from decay. All the centrifuge tubes, tips should be autoclaved to ensure DNase and RNase free.
5. Separate laboratory areas rigorously and perform the procedures in the predefined areas. To avoid cross contamination, all materials used in their designated area should not be moved or used in other areas. False positive results can be caused when cross contamination is not controlled during specimen preparation.
6. All lab workbench and supplies, such as pipettes, centrifuges, PCR cyclers should be disinfected using 1% hypochlorous acid or UV light for 25-30 minutes.
7. After amplification, take out the reaction tubes and seal in a specially designed plastic bag to dispose in a designated area.
8. The test specimens involved in this kit should be considered as infectious substances, and their treatment and handling must meet the relevant regulations of the *General Guidelines for Biosafety of Microbiology and Biomedical Laboratories* and the *Medical Waste Management Regulations* issued by of the Ministry of Health.

【Reference Ct Value for Positive Result】

The reference Ct value to determine target gene as positive is set at 38. The internal control for Ct value is 38.

【Interpretation for Test Results】

1. If a typical sigmoidal curve is observed in Cy5 channel of the specimen and Ct ≤ 38, the results can be determined as the table below.

FAM channel / VIC channel	Ct≤38	38<Ct≤40 (in a sigmoidal shape)	Ct>40
Ct≤38	Positive	Positive	Suspected positive
38 < Ct ≤ 40 (in a sigmoidal shape)	Positive	Suspected positive	Suspected positive
Ct＞40	Suspected positive	Suspected positive	Negative

For specimens tested as positive, when the Ct values of a target gene are between 38 and 40, it is necessary to observe if the amplification curve of the target gene is in sigmoidal shape. If not, the specimen should be regarded as suspected positive.

The suspected positive specimens should all be double-checked. If the double-checked result shows both amplification curves of FAM and VIC channels are in a sigmoidal shape with Ct vales no higher than 40, it is positive, If the Ct values of both channels are higher than 40, it is negative. Other results are suspected positive and it is suggested to conduct the detection again.

2. If the Ct value of Cy5 channel is higher than 38 without showing an apparent sigmoidal amplification curve, the causes can be listed as following:

   1) PCR inhibitors exist in the specimen. It is suggested to dilute the specimen before test.

   2) The operation of nucleic acid extraction is flawed. It is suggested to repeat nucleic acid extraction for the test.

   3) Eligible specimens were not obtained in the processing procedures or specimens have been degraded during transportation and storage. It is suggested to perform sampling again.

【Limitations of Detection Method】

1. The test result is provided for reference only in clinical practice, but it cannot be the sole evidence for diagnosis.
2. Negative results can be caused by low quality of RNA extracted from the specimens, improper storage conditions of extracted RNA solution, inappropriate storage period, inhibitors in the specimen, nucleic acid degradation, *etc*.
3. False negative or false positive results are likely to be caused by inappropriate collecting, transportation and handling of specimens, or unsuitable experiment operation and environment. Other clinical observations and relative information should be combined for determination. Conduct the detection again when necessary.
4. False negative results may occur by sequence changes of target sequence of SARS-CoV-2 due to mutations or other reasons.

【Product Performance】

1. Minimum detection limit: 500 copies/ml.
2. Accuracy

   The positive detection rate should be 100%. The negative detection rate for negative control should be 100%.
3. Analytical Specificity

   The SARS-CoV-2 was compared with four human coronaviruses of HCoV-HKU1, HCoV-229E, HCoV-OC43 and HCoV-NL63. The results indicate that the SARS-CoV-2 is tested as positive and the other four coronaviruses are tested as negative. The reference standards with and without mucin both should be tested as positive. The negative samples should be tested as negative.
4. Precision

   Quantitative fluorescence PCR is used with negative samples, limited positive samples, and strong positive RNA samples. The results indicate that the negative detection rate of the negative samples is 100%; the positive detection rate of the strong positive samples and the limited positive samples are 100% and ≥95% respectively.

【Precautions】

1. Please read the manual carefully before test and follow the protocol strictly.
2. Set both positive and negative controls for each test.
3. Test analysts should be trained by professionals and must perform operation in labs following safety guidelines and wear personal protective equipment.
4. The kits should avoid light for storage to protect the fluorophore from decay. All the centrifuge tubes, tips should be autoclaved to ensure DNase and RNase free.
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【References】

1. X Tang, C Wu, *et al.* On the origin and continuing evolution of SARS-CoV-2. National Science Review. 2020
2. Guidelines for Laboratory testing for COVID-19 (the fifth edition)

【Symbols and Interpretations】

				
For in vitro diagnostic use only	Attention, see instruction for use	Do not use if package is damaged	Limiting temperature	Do not reuse
				
Afraid of the sun	Manufacturer	Date of production	Validity	Batch code
				
Conformity of European	Authorized Representative	Tests per kit	Keep dry	Catalog

【Basic information】

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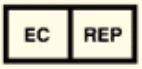

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