- After amplification, take out and put the reaction tubes/plates in a sealable plastic bag, and discard it in a designated place;
- Do not loosen the lid after amplification in case of aerosol contamination;
- Used tips shall be put directly into waste tanks containing 10% sodium hypochlorite and disposed together with other wastes;
- The experiment bench and equipment shall be decontaminated regularly by wiping with 75% alcohol and radiating with UV lamps;
- Avoid cross-contamination between reagents;
- Avoid reuse.

Basic information

Symbol	Meaning	Symbol	Meaning
IVD	In vitro diagnostic medical device		Storage temperature limit
	Manufacturer		Expiration date
	Date of Manufacture		Do not reuse
LOT	Batch code		Consult instructions for use
EC REP	Name and Address of European Union Representative	CE	CE Symbol

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CE EC REP

SUNGO Europe B.V.
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Manufacturing date and expiration date: view on label

VERSION:3.8

Instructions for Use

Name: SARS-CoV-2 & Influenza A/B Nucleic Acid Test

Packaging Specifications

Reagent Quantity	Catalog No.
48 tests/Box	CW3136S
96 tests/Box	CW3136M
960 tests/Box	CW3136L

Intended Use

The SARS-Cov-2 & Influenza A/B Nucleic Acid Test is a multiplex real-time RT-PCR assay intended for simultaneous qualitative detection and differentiation of SARS-CoV-2, influenza A (IFA), and influenza B (IFB) virus RNA in upper or lower respiratory specimens (such as nasopharyngeal and oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate) collected from individuals suspected of respiratory viral infection consistent with SARS-CoV-2, IFA or IFB by their healthcare provider. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, IFA or IFB can be similar.

The SARS-Cov2 & Influenza A/B Nucleic Acid Test is intended for use by trained laboratory personnel who are proficient in performing real-time RT-PCR assays. The laboratories shall have reasonable biosecurity precautions taken and protective procedures in place. The test should only be performed in laboratories that follow safety practices according to the applicable Biosafety Regulations in Microbiological and Biomedical Laboratories.

Positive results indicate active infection of SARS-CoV-2, IFA or IFB, but do not preclude co-infection with other pathogens. Clinical correlation with patient history and other diagnostic information is necessary when diagnosing patient infection status. The agent detected may not be the definite cause of disease.

Negative results do not rule out SARS-CoV-2, IFA or IFB infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history and epidemiological information.

Test Principle

The SARS-Cov2 & Influenza A/B Nucleic Acid Test is a multiplex real-time reverse transcription polymerase chain reaction (RT-PCR) assay run in a single well/vessel, designed for simultaneous detection and differentiation of RNA of SARS-CoV-2, IFA and IFB viruses.

It contains five primer/probe sets in total, of which, two target the ORF1ab non-structural region and N gene that are unique to SARS-CoV-2, one targets a conserved region of the M1 gene of IFA, one targets the NS2 gene of IFB, and one targets the human RNase P gene and acts as the internal process control (IPC). The IPC is to assess the RT-PCR processes including sample collection, processing and nucleic acid amplification.

Reagents

Labels	Main Contents	CW3136S 48 tests/Box	CW3136M 96 tests/Box	CW3136L 960 tests/Box
SF Reaction Mixture	Reverse transcriptase, hot start DNA polymerase; primer and probe sets of SARS-CoV-2, IFA, IFB and IPC; the reaction buffer, etc. (Lyophilized powder)	48 tubes	96 tubes	960 tubes
SF Reaction Dissolvent	Tris, EDTA, etc.	1tube (600μL/tube)	1tube (600μL/tube)	10tubes (600μL/tube)
SF Positive Control	Gene targets of SARS-CoV-2, IFA, IFB and IPC (Lyophilized powder)	1 tube	1 tube	10 tubes
SF Negative Control	Gene targets of IPC (Lyophilized powder)	1 tube	1 tube	10 tubes
SF Control Dissolvent	Tris, EDTA, etc.	1tube (1mL/tube)	1tube (1mL/tube)	10tubes (1mL/tube)

Materials and Equipment Required but Not Provided

RNA extraction or purification kits (Recommended product: CWY070 of Jiangsu CoWin Biosciences Co., Ltd. or verified products from other manufacturers), Medical Gloves, Medical masks, Centrifuge, Vortex mixer, Pipettes and PCR Consumables.

The Storage Condition and The Shelf Life

Manufacturing and expiration dates of the SARS-Cov2 & Influenza A/B Nucleic Acid Test are printed on the packing box.

The kit can be stored at 2-8℃ for 12 months before use.

The kit can be transported at room temperature within a month.

Limitations of the Test

Test results and interpretations are only for clinical reference and should be analyzed in combination with patient history and clinical symptoms. The optimal sample type and the time to reach maximum titer after infection have not been verified. Therefore, collecting samples from the same patient at different times and of multiple types may help avoid false negative results. Test results can be affected by collecting, disposing, transporting and storage of specimens. Any errors of these processes will result in false negative results. Sequence mutations at primer or probe sites used in the test may cause false negative results. Cross-contamination during sample processing may lead to false positive. A negative result only indicates the viral concentration of the sample is lower than the detection limit of the test, thus an infection cannot be excluded.

Product Performance

1. Tested with ten positive standards (P1~P10), and the compliance rate is 100%;
2. Tested with twelve negative standards (N1~N12), and the compliance rate is 100%;
3. The Minimum Detection Limit: 300 copies/ml for SARS-Cov-2, 500 copies/ml for IFA and 500 copies/ml for IFB; tested the detection limit standard (L), and the positive detection rate is ≥ 95%;
4. Tested with the precision standards of the enterprise (S1, S2), and all are positive with the coefficient of variance (CV) of Ct values under 5%;
5. Specificity: There is no cross-reactions with influenza C viruses, coronavirus HcoV-229E, HcoV-HKU1, HcoV-OC43, HcoV-NL63, SARS, MERS, Respiratory syncytial virus A/B, Parainfluenza virus 1/2/3/4, Adenovirus type 1/2/5, Enterovirus A/B/C/D, Human interstitial pneumovirus, Epstein-Barr virus, Cytomegalovirus, Chlamydia pneumoniae, Mycobacterium tuberculosis, Streptococcus pneumoniae, Mycoplasma pneumoniae.

Precautions

- This test is an in vitro diagnostic reagent product that shall be used by trained and experienced testers. Please read the instructions thoroughly before conducting the experiment.
- All used consumables shall be disposed after sterilization.
- Sample processing shall be carried out in biosafety cabinets to protect the testers and to prevent environmental contamination.
- When adding samples, make sure the tips of liquid handlers are completely submerged into the reaction mixture; After adding samples, cover tube lids as soon as possible and spin the tube;

Negative result

When all controls exhibit the expected results, a specimen is considered negative if all viral targets' cycle threshold curves (IFA, IFB and SARS-Cov-2) DO NOT cross the threshold line BEFORE 37.00 Ct (< 37.00 cycles) AND the IPC DOES cross the threshold line at <37.00 Ct.

Invalid result

When all controls exhibit the expected results, and no curves cross the threshold line BEFORE 37.00 Ct (< 37.00 cycles) for IFA, IFB, SARS-Cov-2, and IPC, then the result is invalid. Repeat testing of specimen nucleic acid and/or re-extract and repeat RT-PCR. If the specimen remains invalid upon retest, collection of a new specimen and subsequent testing should be considered.

IFA (FAM) Result	IFB (VIC) Result	SARS-Cov-2 (ROX) Result	IPC (CY5) Result	Interpretation	Report	Actions
+	-	-	+ or -	IFA RNA detected	Positive for influenza A	Report results to sender
-	+	-	+ or -	IFA RNA detected	Positive for influenza B	Report results to sender
-	-	+	+ or -	SARS-Cov-2 RNA detected	Positive for SARS-Cov-2	Report results to sender
-	+	+	+ or -	IFB and SARS-Cov-2 RNA detected	Positive for influenza B and SARS-Cov-2	Report results to sender
+	+	-	+ or -	IFA and IFB RNA detected	Positive for influenza A and Influenza B	Report results to sender
+	-	+	+ or -	IFA and SARS-Cov-2 RNA detected	Positive for Influenza A and SARS-Cov-2	Report results to sender
+	+	+	+ or -	IFA, IFB and SARS-Cov-2 RNA detected	Positive for influenza A, influenza B and SARS-Cov-2	Report results to sender
-	-	-	+ (<37 CT)	Not Detected	Negative	Report results to sender. Consider testing for other respiratory viruses
-	-	-	- (<37 CT)	Invalid result	Invalid	Consider repeat of extraction and/or RT-PCR or collecting a new specimen

a. Laboratories should report their diagnostic result as appropriate and in compliance with their specific reporting system.

b. Optimum specimen types and timing for peak viral levels during infections caused by 2019-nCoV have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the virus. The possibility of a false negative result should especially be considered if the patient's recent exposures or clinical presentation suggest that 2019-nCoV infection is possible, and diagnostic tests for other causes of illness (e.g., other respiratory illness) are negative. If 2019-nCoV infection is still suspected, re-testing should be considered in consultation with public health authorities.

Applicable Equipment

The test is applicable to the Applied Biosystems™ 7500 Real-Time PCR Instrument, the Applied Biosystems™ 7500 Fast Real-Time PCR Instrument, and the Bio-Rad CFX96 real-time PCR thermocycle.

Acceptable Specimens

The types of specimens for the test include nasopharyngeal and oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate from patients or suspected patients. Collected specimens should be transported and stored according to the specimen collection vessel instructions.

For nasopharyngeal swabs or oropharyngeal swabs, we strongly recommend collecting sample with Viral Sample Preservation Solution of Jiangsu CoWin Biosciences Co., Ltd. (CW3129), thus samples can be amplified directly without RNA extraction. Virus preservation solution of other manufacturers (main component is Hanks preservation solution or normal saline) are compatible with our kit to perform the direct amplification procedure, but should be verified before detection.

Preparation before Detection

The function of the dissolvent is to redissolve lyophilized powder. Incorrect use of the dissolvent may lead to the failure of the experiment. Before performing the assay, SF Positive Control (Lyophilized powder) and SF Negative Control (Lyophilized powder) need to be reconstituted as follows:

- Centrifuge SF Positive Control (Lyophilized powder) and SF Negative Control (Lyophilized powder) at 12000 rpm for 2 minutes;
- Carefully open the lid of SF Negative Control (Lyophilized powder) and add 500μL SF Control Dissolvent;
- Shake and mix several times until the lyophilized powder is completely dissolved;
- Give the tube a short spin using a centrifuge;
- Carefully open the lid of SF Positive Control (Lyophilized powder) and add 500μL SF Control Dissolvent;
- Shake and mix several times until the lyophilized powder is completely dissolved;
- Give the tube a short spin using a centrifuge.

Detection Methods

Step 1. Sample processing

- Extract the RNA of collected specimens following the instructions of the RNA extraction or purification kit used.

- If the specimens are nasopharyngeal swabs or oropharyngeal swabs collected by the virus sample preservation solution (Viral Sample Preservation Solution of Jiangsu CoWin Biosciences Co., Ltd. (CW3129) or Virus preservation solution which main component is Hanks preservation solution or normal saline), those samples can be amplified directly without RNA extraction.

Step 2. Preparation of Amplification Reagents

- Determine the number of reaction tubes to be used according to the number of samples (The number of reaction tubes is N = n + 2, where n is the number of samples to be tested, and 2 accounts for the negative control and the positive control.) ;
- Take out appropriate numbers of SF Reaction Mixture and SF Reaction Dissolvent from the test box;
- Shake SF Reaction Dissolvent sufficiently, followed by a quick spin using a centrifuge at a low speed;
- For SF Reaction Mixture (8-Tube Strip), first spin 30 seconds using a centrifuge, then open the lid gently to avoid any losses of mixture powder, and add 5μL SF Reaction Dissolvent to each tube.

Step 3. Add the Templates

20 μL templates are added to reconstituted SF Reaction Mixture. According to different template types, the adding operations are carried out as follows:

- When the templates are positive or negative controls
- Add 20μL reconstituted SF Positive Control or SF Negative control.
- When the templates are extracted RNA
- Add 20μL extracted RNA sample.
- When the templates are specimens collected with virus sample preservation solutions (Viral Sample Preservation Solution of Jiangsu CoWin Biosciences Co., Ltd. or Virus preservation solution of other manufacturers with Hanks preservation solution or normal saline as the main component)
- Add 20μl virus sample.
- Cap the tubes tightly, then shake and mix thoroughly by vortex, centrifuge briefly.

Step 4. Amplification

Put reaction tubes into the real-time PCR thermocycler and set the cycle program as follows:

Step	Cycles	Temperature (°C)	Time (min:sec)
1	1	55	01:00
2	1	96	00:20
3	45	96	00:05
		60	00:30

* Fluorescence signals are collected.

The fluorescence channels are assigned as:

- FAM - IFA;
- VIC - IFB;
- ROX - SARS-CoV-2;
- CY5 - Internal Process Control.

The reaction volume is set as 25 μL per tube. When using the ABI7500, select ‘Quencher’ and ‘Passive reference’ settings as "none".

Cut-off values

The cut-off value is 37.0 and was determined by limited-dilution testing.

Interpretations of Test Results

After reactions are completed, the instrument automatically saves the reaction data, determines the Start and the End of the Baseline, and determines Threshold values after analyzing amplification curves. These parameters are user-adjustable; for example, the Start of the Baseline is usually set between 3 and 15, the End between 5 and 20, and the Threshold is usually set at or slightly above the amplification curve of the PCR-negative control.

The negative and positive controls in the SARS-Cov2 & Influenza A/B Nucleic Acid Test must meet the following criteria to validate an experiment, otherwise the experiment will be deemed invalid.

Criteria for a Valid Experiment	FAM (IFA)	VIC (IFB)	ROX (SARS-CoV-2)	CY5 (IPC)
SF Positive Control	Ct<37.0	Ct<37.0	Ct<37.0	Ct< 37.0
SF Negative Control	no Ct or Ct≥37	no Ct or Ct≥37	no Ct or Ct≥37	Ct<37.0

When all criteria for the positive and negative controls are met, test results are interpreted as follows:

Positive result

When all controls exhibit the expected results and one or more of the viral targets (IFA, IFB, and/or SARS-Cov-2) DOES cross the threshold line BEFORE 37.00 Ct then the specimen is considered positive for those virus(es). Multiple viruses may be detected in a single specimen.