SARS-CoV-2 Antigen Rapid Test Kit

Instruction for Use

FOR IN VITRO DIAGNOSTIC USE

This instruction for use (IFU) must be read carefully prior to use. Instruction for use must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions for use.

PRODUCT NAME

SARS-CoV-2 Antigen Rapid Test Kit

PACKING SPECIFICATION

1 test/kit, 2 tests/kit, 5 tests/kit, 10 tests/kit ,25 tests/ kit, 50 tests/ kit

INTENDED PURPOSE OF THE DEVICE

This SARS-CoV-2 Antigen Rapid Test Kit is only used for rapid in vitro qualitative detection of nucleocapsid protein (N protein) from SARS-CoV-2 antigen in human nasopharyngeal swabs, anterior nasal swab or posterior oropharyngeal saliva within 5 days after clinical symptoms.

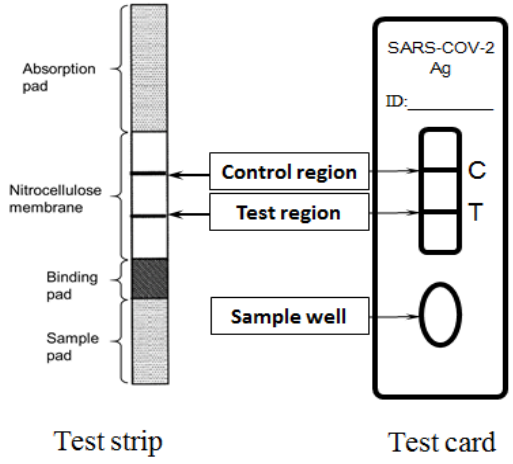
This test is intended for clinical laboratories, medical institutions, or real-time inspection by professional medical staff only.

TEST PRINCIPLE

The SARS-CoV-2 Antigen Rapid Test Kit is a colloidal gold enhanced double antibody sandwich immunoassay for the qualitative determination of nucleocapsid protein (N protein) from SARS-CoV-2 antigen. The test card consists of a plastic housing and a test strip which is composed of absorption pad, nitrocellulose membrane (NC membrane), binding pad and sample pad.The test region (T) of the nitrocellulose membrane was immobilized with mouse anti SARS-CoV-2 antibody 1 (Ab1), and the control region (C) of the nitrocellulose membrane was immobilized with Goat anti chicken IgY antibody (GAC). At the same time, the binding pad of the test card was fixed with mouse anti SARS-CoV-2 antibody 2 (Ab2) and chicken IgY (CIgY) which were both labeled by colloidal gold.

When the sample contains SARS-CoV-2 antigen is added to the sample well of the detection card, it will react with Ab2 to form complexes “antigen-Ab2-colloidal gold”. When the complexes migrates along the membrane, it can be captured by the Ab1 immobilized in the test region to form complexes “Ab1-antigen- Ab2-colloidal gold”, and a purple band appeared in the test region. The absence of this colored band in the test region suggests a negative result. The chicken IgY labeled with colloidal gold on the binding pad will migrate along the nitrocellulose membrane with the sample, and be captured by GAC antibody in the control region to form a GAC-CIgY-colloidal gold complex, thus a purple band always appears in the control region regardless of the sample contains SARS-CoV-2 or not.

The test results of this kit cannot be used as the only basis for the diagnosis and exclusion of pneumonia caused by SARS-CoV-2. Positive results from the test need further analysis with patient clinical history and other diagnostic information to determine patient infection status. Positive results can only serve as a reference guide for clinical diagnosis. The test results only reflect the current state of the sample. Negative results cannot exclude SARS-CoV-2 infection and should NOT be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. The laboratory testing of SARS-CoV-2 should meet the requirements of the Laboratory testing for SARS-CoV-2 in suspected human cases and other requirements, and pay attention to the biosecurity.



REAGENTS AND MATERIALS SUPPLIED

1. Main components:

Specification / component	1 test/kit	2 tests/kit	5 tests/kit
Test card	1	2	5
Sample extraction solution	500μL*1	500μL*2	500μL*5
Tube & Dripper	1	2	5
Manual	1		

Specification / component	10 tests/kit	25 tests/kit	50 tests/kit
Test card	10	25	50
Sample extraction solution	500μL*10	500μL*25 or 20mL*1	500μL*50 or 20mL*2
Tube & Dripper	10	25	50
Manual	1		

2. Main ingredients of the Test card

SARS-CoV-2 antibody	Coated in the Test region on NC membrane
Goat anti-Chicken IgY polyclonal antibody	Coated in the control region on NC membrane
SARS-CoV-2 antibody, Chicken IgY, Colloidal gold conjugate	Coated in the conjugate pad
Other test device supports	/

3. Main ingredients of the sample extraction solution

• Surfactant, preservative and Tris-HCl buffer solution

Note: The components in different batches of the kit cannot be mixed.

REQUIRED MATERIALS

1. Nasopharyngeal swab:
2. Oropharyngeal swab:
3. Nasal swab

Note: Swabs are required and provided in the kit. Any one type out of the above 3 type swabs for selection.

MATERIALS REQUIRED BUT NOT PROVIDED

• Timer

STORAGE AND STABILITY

1. Kits shall be stored at 2°C ~30°C in a cool, dark, and dry place, valid refer to expiry date. Kits is not recommended to be stored under 2℃, and expired products shall **NOT** be used.
2. The test card should be in aluminum foil pouch before opening and used within 1 hour in the specified environment (temperature 2°C~35°C, humidity 40%~60%) after opening.
3. The buffer should be used immediately after dropping into the dropper.
4. MFD date and EXP date: marked on the label.

SPECIMEN REQUIEMENTS

1. Nasopharyngeal Swab Sample:

To collect a nasopharyngeal swab sample, carefully insert the swab into the nostril that presents the most secretion under visual inspection. Keep the swab near the septum floor of the nose while gently pushing the swab into the posterior nasopharynx. Rotate the swab several times then remove it from the nasopharynx.

2. Anterior Nasal Swab Sample:

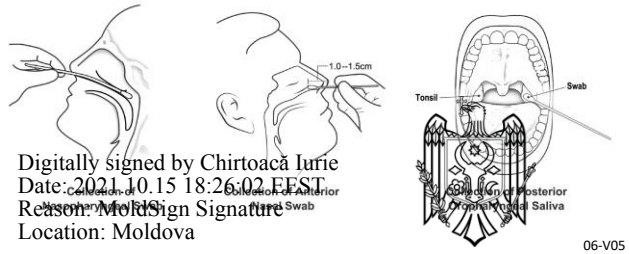
To collect an anterior nasal swab, insert the swab about 1 cm (0.5 inch) to 1.5 cm (¾ of an inch) into the nostril, and firmly sample the nasal membrane by slowly rotating the swab in a circular path against the inside of your nostril at least 4 times and leaving in place for 10 to 15 seconds. Be sure to collect any nasal drainage that may be present on the swab. Gently remove the swab, and sample the other nostril with the same swab as described above.

3. Posterior oropharyngeal saliva:

To collect a posterior oropharyngeal saliva sample, carefully insert the swab completely into the oropharyngeal swelling, centering on the red part of the throat wall, upper jaw, and tonsils, wipe and rotate 3 times with moderate force, and remove the swab avoid touching the tongue.

4. The samples should be used as soon as possible after collection. The samples can keep stable within 1 hour before mixing with sample extraction solution provided with the kit, once mixed with the sample extraction solution, they can cause the virus to become unstable, so no matter how long they are stored before mixing, it should be used within 30 minutes after mixing.

5. Samples should not be inactivated.



TEST PROCEDURE

NOTE: Do not open the pouch until you are ready to perform a test, and the single-use test is suggested to be used under low humidity (RH≤60%) environment within 1 hour.

Allow all kit components and specimens to reach room temperature between 15°C~30°C prior to testing. Identify the test card for each specimen and paste the ID number of tested person on the blank area on the shell of the test card, and the tester can create the corresponding record files on papers.

Sample processing:

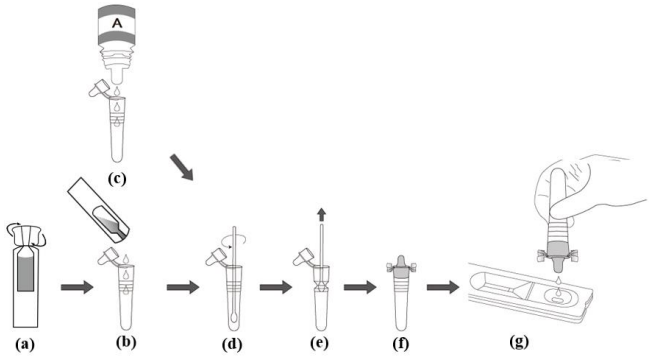
Elute swab with Sample extraction solution

1a. When the specification of sample extraction solution is 500μL, twist off the tip of the sample extraction solution (a), and squeeze all the solution into an extraction tube (b).

1b. When the specification of sample extraction solution is 20mL, add 500μL (~15 drops) of sample extraction solution into the extraction tube (c)

2. Completely immerse the swab tip into the sample extraction solution and mix the solution by rotating the swab forcefully against the side of the tube at least 10 times (d). Squeeze the tube 5 times by hand to ensure that the sample on the swab is fully eluted into the sample extraction solution (e).

3. Squeeze the swab tip along the inner wall of the extraction tube to keep the liquid in the tube as much as possible. Discard the swab and cover the dripper head to mix the liquid thoroughly (f).



Test operation

Before performing the test, you must read the instruction manual of the product carefully, and please allow the test cards and sample extraction solution to equilibrate at room temperature (15℃~30℃) before the test. Do not perform the test until the reagents were equilibrated to room temperature to avoid affecting the accuracy of the test results.

1. Remove the test card from the foil pouch and place on a clean and dry surface. Dispense 80μL (3 drops) of the specimen into the sample well on the card (g).

2. Interpret the test results at 15~20 minutes. Do not interpret the results after 20 minutes.

3. Discard used test tubes and test cards in suitable biohazards waste container.

INTERPRETATION OF TEST RESULTS

Positive: Both purplish test band and purplish control band appear on the membrane.

Negative: Only the purplish control band appears on the membrane. The absence of a test band indicates a negative result.

Invalid: There should always be a purplish control band in the control region regardless of test result. If the control band is not seen, it indicates an incorrect operation process, that the kit has deteriorated or damaged, or the antigen content in the specimen is too high. In this case, read the instructions carefully again and dilute the sample to retest with a new test card. If the problem persists, stop using the product of this lot immediately and contact your local supplier.

Note: The purplish band in the test region (T) can show different color shades. However, within the specified observation time, regardless of the color of the test band, even a very weak(light) band should be judged as a positive result. The purplish band in the control region (C) can show different color shades. However, within the specified observation time, regardless of the color of the control band, even a very weak (light) band should be judged as that the test card is valid.

Positive **Negative** **Invalid**

LIMITATIONS

1. The result of the product should not be taken as a confirmed diagnosis and is only for clinical reference. Judgement should be made along with RT-PCR results, clinical symptoms, epidemic condition, and correlated clinical data.
2. In the early stage of infection, the test result may be negative because the SARS-CoV-2 antigen level is too low or antigen has not yet appeared in the sample.
3. Due to the limitation of the detection method, the negative result cannot exclude the possibility of infection. The positive result should not be taken as a confirmed diagnosis. Judgement should be made along with clinical symptoms and other diagnosis methods.
4. This reagent can only detect SARS-CoV-2 antigens in human nasopharyngeal swab, anterior nasal swab and oropharyngeal swab qualitatively. It cannot determine certain antigen content in the samples.
5. The accuracy of the test depends on the sample collection process. Improper sample collection, improper sample transportation and storage, or repeated freezing and thawing of the sample will affect the test results.
6. It is optimum when eluting the swabs with the matched samples extraction solution. Using other diluents may result in erroneous results.
7. The solution and test card must be equilibrated to room temperature (15℃~30℃) before use. Otherwise, the results may be incorrect.
8. Sensitivity may decrease if the sample was not tested directly. Please test the sample as soon as possible.
9. Cross-reactivity may occur due to the N protein in SARS having high homology with the new coronavirus (SARS-CoV-2). However, the interpretation of the results is not affected during seasons without SARS infection.
10. Analysis the possibility of false-negative results:

1) Inappropriate sample collection, using other non-matching extraction solution, an excess of sample transfer time (more than one hour), an excess of the volume of solution added when eluting the swab, non-standardized elution operation, and low virus titer in the sample, these may all lead to false-negative results.
2) Mutations in viral genes may lead to changes in the antigen epitope, leading to false-negative results.

11. Analysis the possibility of false-positive results:

1) Inappropriate sample collection, using other non-matching solutions, and non-standardized elution operation, these may all lead to false-positive results.
2) Cross-contamination of samples may lead to false-positive results.
3) False-negative result from the nucleic acid test.

12. Analysis the possibility of invalid result:

4) If the sample volume is not enough, the chromatography cannot be carried out successfully.
5) The test card would be invalid if the package was broken. The packaging status must be carefully checked before use.

PERFORMANCE CHARACTERISTIC

1. Performance

1.1. The coincidence rate of positive controls
Tested with 5 positive controls (P1-P5), the results were all positive, and the coincidence rate (+ / +) was 5/5.

1.2. The coincidence rate of negative controls
Tested with 10 negative controls (N1-N10), the results were all negative, and the coincidence rate (+ / +) was 10/10.

1.3. Repeatability
Tested with repeatable control (J) for 10 times, the results were all positive and consistent.

1.4. Limit of detection (LOD)
Tested with the inactivated SARS-CoV-2 virus at a concentration of $100TCID_{50}$/mL for 20 times, and the positive detection rate was ≥95% (the coincidence rate (+ / +) was ≥ 19/20).

Note：The LOD of the reagent varies slightly depending on the strains tested, which is a common phenomenon of reagents based on the principle of immunity. When testing different strains, the LOD of this kit is between $40TCID_{50}$/mL to $100TCID_{50}$/mL.

2. Clinical performance

2.1. Clinical performance of all sample types

The SARS-CoV-2 Antigen Rapid Test Kit has tested 300 cases of human nasopharyngeal swab sample, anterior nasal swab sample and posterior oropharyngeal saliva that were RT-PCR positive (the median Ct value of the RT-PCR is 26.73) for SARS-CoV-2 infection and 891 cases of human nasopharyngeal swab sample, anterior nasal swab sample and posterior oropharyngeal saliva that were RT-PCR negative. The test results are shown in the table below:

SARS-CoV-2 antigen test \ RT-PCR test	Positive	Negative	Total
Positive	295	0	295
Negative	5	891	896
Total	300	891	1191

Sensitivity = 295/ (295+5) ×100% =98.33% (95%CI: 95.93% ~ 99.38%)
Specificity = 891/ 891 ×100% =100% (95%CI: 99.46% ~ 100%)
Accuracy = (295+891)/1191 ×100% =99.58%

2.2. Clinical performance of human nasopharyngeal swab

The SARS-CoV-2 Antigen Rapid Test Kit has tested 100 cases of human nasopharyngeal swab sample that were RT-PCR positive (the median Ct value of the RT-PCR is 24.70) for SARS-CoV-2 infection and 603 cases of human nasopharyngeal swab sample that were RT-PCR negative. The test results are shown in the table below:

SARS-CoV-2 antigen test \ RT-PCR test	Positive	Negative	Total
Positive	100	0	100
Negative	0	603	603
Total	100	603	703

Sensitivity = 100/ (100+0) ×100%=100% (95%CI：95.39% ~ 100%)
Specificity = 603/ 603 ×100%=100% (95%CI：99.21% ~ 100%)
Accuracy = (100+603)/703 ×100%=100%

2.3. Clinical performance of human anterior nasal swab

The SARS-CoV-2 Antigen Rapid Test Kit has tested 100 cases of human anterior nasal swab sample that were RT-PCR positive (the median Ct value of the RT-PCR is 26.87) for SARS-CoV-2 infection and 100 cases of human anterior nasal swab sample that were RT-PCR negative. The test results are shown in the table below:

SARS-CoV-2 antigen test \ RT-PCR test	Positive	Negative	Total
Positive	99	0	99
Negative	1	100	101
Total	100	100	200

Sensitivity = 99/ (99+1) ×100% =99.0% (95%CI：93.76% ~ 99.95%)
Specificity = 100/ (0+100) ×100% =100% (95%CI：95.39% ~ 100%)
Accuracy = (99+100)/ 200 ×100% =99.50%

2.4. Clinical performance of human posterior oropharyngeal saliva

The SARS-CoV-2 Antigen Rapid Test Kit has tested 100 cases of human posterior oropharyngeal saliva that were RT-PCR positive (the median Ct value of the RT-PCR is 28.86) for SARS-CoV-2 infection and 188 cases of human posterior oropharyngeal saliva that were RT-PCR negative. The test results are shown in the table below:

SARS-CoV-2 antigen test \ RT-PCR test	Positive	Negative	Total
Positive	96	0	96
Negative	4	188	192
Total	100	188	288

Sensitivity = 96/ (96+4) ×100% =96.0% (95%CI：89.49% ~ 98.71%)
Specificity = 188/ (0+188) ×100% =100% (95%CI：97.50% ~ 100%)
Accuracy = (96+188)/288 ×100% =98.61%

2.5. Patient demographics

The positive results were subdivided according to the age and gender of patients. The results are shown in the following table.

Age	Gender	Comparator Method		
		Total	Positive	Prevalence
＜18	Male	82	3	3.66%
	Female	61	1	1.64%
18-65	Male	418	103	24.64%
	Female	298	66	22.15%
＞65	Male	173	64	36.99%
	Female	159	63	39.62%

3. Cross-reactivity

Cross-reactivity of the kit was evaluated by diluting the microorganisms in the table below to the concentration described in the table below with negative samples, and the results showed no cross-reactivity with the following microorganism. However, in consideration of the homology between the human coronavirus HKU1, MERS coronavirus, SARS-CoV and SARS-CoV-2, cross reactions may still occur when the virus concentration is higher, which is the same with other microorganisms.

No.	Microorganism	Concentration.
1	Human Coronavirus HKU1	10^5 pfu/mL
2	Human Coronavirus OC43	10^5 pfu/mL
3	Human Coronavirus 229E	10^5 pfu/mL
4	Human Coronavirus NL63	10^5 pfu/mL
5	Influenza A H1N1 (2009)	10^5 pfu/mL
6	MERS-coronavirus	10^5 pfu/mL
7	Sars-coronavirus	10^5 pfu/mL
8	Influenza A H3N2	10^5 pfu/mL
9	Influenza B Yamagata	10^5 pfu/mL
10	Influenza B Victoria	10^5 pfu/mL
11	Respiratory syncytial virus A	10^5 pfu/mL
12	Respiratory syncytial virus B	10^5 pfu/mL
13	Adenovirus type 1	10^5 pfu/mL
14	Adenovirus type 2	10^5 pfu/mL
15	Adenovirus type 3	10^5 pfu/mL
16	Adenovirus type 4	10^5 pfu/mL
17	Adenovirus type 5	10^5 pfu/mL
18	Adenovirus type 7	10^5 pfu/mL
19	Adenovirus type 55	10^5 pfu/mL
20	Bordetella pertussis	10^5 pfu/mL
21	Candida albicans	10^5 pfu/mL
22	Legionella pneumophila	10^5 pfu/mL
23	Enterovirus EV71	10^5 pfu/mL
24	Enterovirus CA16	10^5 pfu/mL
25	Enterovirus CA10	10^5 pfu/mL
26	Enterovirus CB5	10^5 pfu/mL
27	Enterovirus CA24	10^5 pfu/mL
28	Enterovirus CB4	10^5 pfu/mL
29	Enterovirus CB3	10^5 pfu/mL
30	Enterovirus CB2	10^5 pfu/mL
31	Enterovirus CB1	10^5 pfu/mL
32	Enterovirus CA6	10^5 pfu/mL
33	EB virus	10^5 pfu/mL
34	Human cytomegalovirus	10^5 pfu/mL
35	Mycoplasma pneumoniae	10^5 pfu/mL
36	Chlamydia pneumonia	10^5 pfu/mL
37	Haemophilus influenzae	10^5 pfu/mL
38	Human Metapneumovirus	10^5 pfu/mL
39	Human Rhinovirus A30	10^5 pfu/mL
40	Human Rhinovirus A31	10^5 pfu/mL
41	Human Rhinovirus A2	10^5 pfu/mL
42	Human Rhinovirus A81	10^5 pfu/mL
43	Human Rhinovirus B52	10^5 pfu/mL
44	Human Rhinovirus B70	10^5 pfu/mL
45	Human Rhinovirus B72	10^5 pfu/mL
46	Metapneumovirus A2	10^5 pfu/mL
47	Metapneumovirus Type B1	10^5 pfu/mL
48	Metapneumovirus Type B2	10^5 pfu/mL
49	Measles virus	10^5 pfu/mL
50	Rubella virus	10^5 pfu/mL
51	Rhinovirus	10^5 pfu/mL
52	Mumps virus	10^5 pfu/mL
53	Boca virus	10^5 pfu/mL
54	Parainfluenza Virus 1-4	10^5 pfu/mL
55	Streptococcus pneumoniae	10^5 pfu/mL
56	Streptococcus pyogenes	10^5 pfu/mL
57	Mycobacterium tuberculosis	10^5 pfu/mL
58	Pneumocystis jirovecii (PJP)	10^5 pfu/mL
59	Staphylococcus aureus	10^5 pfu/mL
60	Staphylococcus epidermidis	10^5 pfu/mL

4. Interference Substances

Substances at the following concentration do not interfere with the test results:

No.	Substances	Concentration
1	Ibuprofen	1mg/mL
2	Tetracycline	3μg/mL
3	Chloramphenicol	3μg/mL
4	Erythromycin	3μg/mL
5	Tobramycin	5%
6	Throat spray (Menthol)	15%
7	Mupirocin	10mg/mL
8	Throat lozenge (Menthol)	1.5mg/mL
9	Oseltamivir	5mg/mL
10	Naphthoxoline hydrochloride nasal drops	15%
11	Mucin	0.50%
12	Fisherman's Friend	1.5mg/mL
13	Compound Benzocain Gel	1.5mg/mL
14	Cromoglycate	15%
15	Phenylephrine Hydrochloride	15%
16	Afrin (Oxymetazoline)	15%
17	Fluticasone propionate spray	15%
18	Whole Blood	4%
19	Chloraseptic (Menthol/Benzocaine)	1.5 mg/mL
20	Naso GEL (NeilMed)	5% v/v
21	CVS Nasal Drops (Phenylephrine)	15% v/v
22	Zicam	5% v/v
23	Homeopathic (Alkalol)	1:10 dilution
24	Sore Throat Phenol Spray	15% v/v
25	Tamiflu (Oseltamivir Phosphate)	5 mg/mL

PRECAUTIONS

1. The reagent is disposable for in vitro diagnostic use only.
2. The test results cannot be used as the basis for the diagnosis and exclusion of pneumonia caused by SARS-CoV-2.
3. The operation should be carried out strictly according to the instructions. Do not use expired or damaged products.
4. Reagents should be used as soon as possible （within 1 hour）after removal from aluminum foil bags to avoid long-time air exposure and dampness that might affect the test results.
5. Do not use samples that have been placed for too long or contaminated.
6. Please operate under the laboratory testing procedures for infectious diseases. Waste after use should be treated as infectious substances and should not be discarded at will.
7. Incorrect operation may affect the accuracy of the results, such as insufficient sample mixing, insufficient sample amount, wrong detection time, etc.
8. Components in different batches should not be mixed.
9. There should be appropriate biosafety assurance procedures for those substances containing suspected sources of infection. The following are relevant considerations:

1) Handle samples and reagents with gloves;
2) Do not suck samples with your mouth;
3) Considering that the tester's hands may be infected with a virus DURING the test, do not touch vulnerable areas such as the mouth, nasal cavity or eyeballs with your hands during the test. Therefore, smoking, eating, drinking, putting on makeup or handling contact lenses cannot be performed while handling samples and reagent;
4) Disinfect the spilled sample or reagent with disinfectant;
5) Disinfect and treat all samples, reagents, and potential pollutants according to relevant local regulations;
6) Each component of the reagent remains stable until the expiry date under proper handling and storage conditions. Do not use the expired reagent kit.

APPLICABLE SYMBOLS

Symbol	Used for	Symbol	Used for
	Use-by date		Consult instructions for use
LOT	Batch code	IVD	In vitro diagnostic medical device
	Temperature limit		Manufacturer
CE	CE mark	EC REP	Authorized representative in the European Community
Σ	Tests per kit	REF	Reference
2	Please don't reuse it		Biological risks
	Don’t use the product when the package is damaged		Keep dry
	Date of manufacture		

【BASIC INFORMATION】

Manufacturer: Triplex International Biosciences (China) Co., LTD.
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【INSTRUCTION APPROVAL AND REVISION DATE】

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