SARS-CoV-2 Antigen RAPID TEST Kit

(Fluorescence Immunochromatography)

Instruction for Use

CE

For in vitro diagnostic use only
Store at 2°C **-30**°C

CONTENT

1. INTENDED USE

The novel coronaviruses belong to the β genus. COVID-19 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the novel coronavirus are the main source of infection: asymptomatic infected people can also be an infectious source. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.

The genome of coronavirus encodes spike protein, envelope protein, membrane protein and nucleocapsid. In the process of viral assembly, N protein binds to viral RNA and leads to the formation of spiral nucleocapsid. N protein is a highly immunogenic phosphoprotein, which is related to viral genome replication and cell signaling. Because of the conserved sequence of N protein, detection of SARS-CoV-2 N protein is of great clinical significance.

This rapid kit is used for qualitative detection of SARS-CoV-2 nucleocapsid protein antigen (hereinafter referred to as “SARS-CoV-2” N antigen) in human serum.

2. TEST PRINCIPLE

This rapid kit uses fluorescence immunochromatography method to detect SARS-CoV-2 N antigen in human serum. Apply the sample to be tested into the sample hole of the test cassette, the test analyte in the sample will form a complex with the fluorescent microsphere-labelled antibody on the binding pad under chromatography, and the complex will continue to be chromatographed on the nitrocellulose membrane until detection Line (T line), then captured by the detection line antibody. The unbound microspheres are chromatographed to the quality control line (C line) and captured by the antibody on the quality control line. A fluorescent flashlight with a wavelength of 365nm was used to illuminate the observation window. The red fluorescent signal on the T line and the C line at the same time indicates that the SARS-CoV-2 N antigen is positive; only the C line and no T line indicates that the SARS-CoV-2 N antigen is negative. If the C line does not appear, the test result is invalid, and this sample needs to be tested again with another test cassette.

3. KIT COMPONENTS

25 Test Cassettes;

1 Instruction For Use

4. WARNINGS AND PRECAUTIONS

4.1. For prescription use only. For in vitro diagnostic use only. Do not use after expiration date.

4.2. Samples for human serum should be considered as potentially infectious. Operators should wear protective clothing, masks, gloves and take other appropriate safety precautions to avoid or reduce the risk of infection.

4.3. This test should be performed at 18 to 30°C (64 to 86°F). Ensure that the kit is brought to operating temperature before performing testing.

4.4. Follow the instructions for use carefully. Reliability of assay results cannot be

guaranteed if there is any deviation from the instructions inserted in this package.

4.5. Professionals must handle the potentially contaminated materials safely according to local requirements.

4.6. Do not smoke, drink, eat, or use cosmetics in the working area. Wear Personal Protective Equipment and disposable gloves when working with samples and reagents. Wash hands after operations.

4.7. Wipe and wash any splashed sample with highly effective disinfectant. Avoid splashing and the formation of aerosols.

4.8. Use a new clean disposable sample tip for each sample to avoid cross contamination.

4.9. Don't look directly into the flashlight.

4.10.Decontaminate and dispose of all samples, reaction kits, and potentially contaminated materials as if they were infectious waste, in a biohazard waste container.

4.11.Once the cassette is removed from the pouch, Use the cassette as soon as possible to avoid being humidified. The cassette is sensitive to humidity as well as to heat.

4.12. Do not use the cassette if the pouch is damaged or the seal is broken.

4.13. The test card cannot be reused..

5. STORAGE CONDITIONS AND SHELF LIFE

The test cassette is stored at 2℃～30℃, and the shelf life is 12 months. The test cassette sealed inside the aluminium foil bag shall be used within 1 hour after opening.

6. APPLICABLE INSTRUMENTS

365nm wavelength fluorescent flashlight.

7. SAMPLE REQUIREMENTS

7.1 Applicable to human serum.

7.2 For serum samples, the samples shall be tested immediately after collection. Serum samples can be stored for 5 days at 2-8°C. If long-term storage is required, it should be stored at -20°C. Serum specimens can be subjected to a maximum of 3 freezing / thawing cycles.

7.3 Let the samples reach room temperature and mix well before testing. When there are visible particles in the sample, it should be centrifuged before the test to remove the precipitate.

7.4 If there is a lot of lipid (Triglyceride concentration over 37 mmol/L), hemolysis or turbidity in the sample, please do not use the sample to avoid affecting the result interpretation.

8. MATERIALS REQUIRED BUT NOT PROVIDED

- Sample vortex mixer
- 10-100μl pipette and tips

- Test tubes
- Sample collection tubes
- Timer
- 365nm wavelength fluorescent flashlight.

9. TEST PROCEDURES

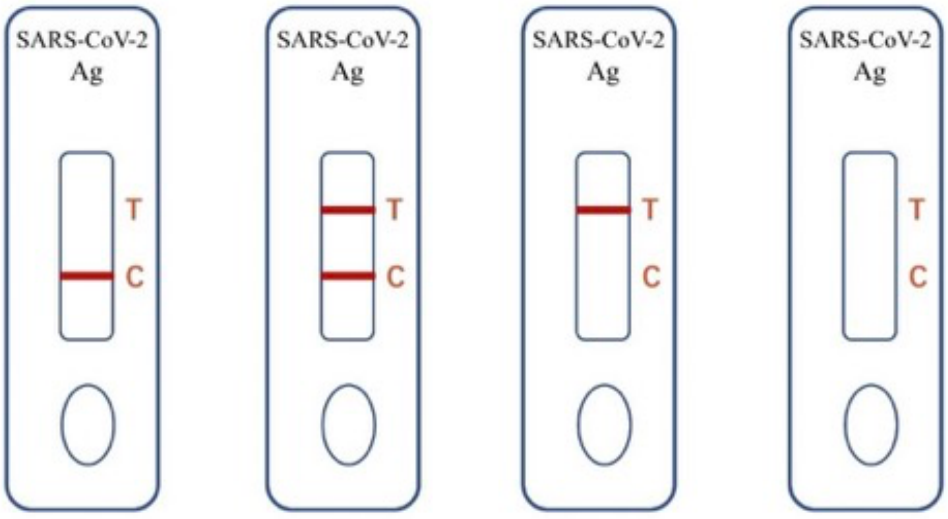
Step 1: Take out the sample to be tested and let it reach room temperature. Mix the sample well before testing.

Step 2: Tear the aluminium foil bag to open, take out the detection cassette and place it on the horizontal surface.

Step 3: Write the sample number on the test cassette.

Step 4: Take 80μL of the sample to be tested with the pipette into the sample hole on the test cassette immediately, and ensure that there is no bubble during the operation.

Step 5: Read the test results between 15 and 20 minutes.Observe the test result immediately (within 10 seconds) under the illumination of a fluorescent flashlight. (Long-time exposure under fluorescent flashlight will cause the diminishing of fluorescence signal, which will affect the interpretation of the result.)



Negative result | Positive result | Invalid result | Invalid result

10. INTERPRETATION OF THE RESULTS

10.1. Due to the complex structure of bioactive substances in samples and the difference of antigen antibody specificity, the possibility of false positive results cannot be completely ruled out by using this kit. If the test results are inconsistent with the clinical indications, other appropriate test methods should be used for confirmation.

10.2. SARS-CoV-2 N protein novel coronavirus nucleocapsid protein, N antigen positive is

an important sign of SARS-CoV-2 infection, indicating that there is SARS-CoV-2 infection. But the negative result of SARS-CoV-2 N antigen cannot completely exclude the infection of SARS-CoV-2. The reason is that when the content of SARS-CoV-2 N antigen in the sample is below the detection limit or anti-N antigen antibody has been produced in the serum, the content of N antigen decrease.

10.3. The test results of this kit are only used as the basis of auxiliary diagnosis. Clinical diagnosis should be combined with clinical symptoms and other diagnostic methods.

11. LIMITATION OF THE PROCEDURES

11.1. Hyperlipidemia, hemolysis samples, samples contaminated with microorganisms, repeated freezing and thawing more than 3 times or samples after heat inactivation may affect the accuracy of the detection and lead to erroneous results.

11.2. Samples with severe jaundice or serious pollution will lead to wrong results.

12. PERFORMANCE CHARACTERISTICS

12.1 Detection limit

LoD: The detection limit of the kit was studied with serum samples added with recombinant N protein, repeat the test 60 times, and the LoD is 3.5 pg/mL.

12.2 Virus Detection Limit:

Dilute the novel coronavirus stock solution (2.0×10^4 TCID50/mL) (IVCAS 6.7512) that has been inactivated at 56 degrees for 30 minutes with negative serum to 200 $TCID_{50}$/mL, 100 $TCID_{50}$/mL, 40 $TCID_{50}$/mL, 20 $TCID_{50}$/ mL, 10 $TCID_{50}$/mL, 5 $TCID_{50}$/mL samples. Each sample was tested 3 times. The test results of samples as below. Set 20 $TCID_{50}$/mL as the LoD of the kit for virus detection. Negative serum added with inactivated virus at a concentration of 20 $TCID_{50}$/mL were tested 20 times for verification. A total of 20 test results were positive. The LoD of detecting the inactivated novel coronavirus sample of this kit is determined to be 20 $TCID_{50}$/mL.

Determination of detection limit of inactivated novel coronavirus

Test Concentration ($TCID_{50}$/mL)	Test times	Test Result
20000	3	3/3 Positive
200	3	3/3 Positive
100	3	3/3 Positive
40	3	3/3 Positive
20	3	3/3 Positive
10	3	1/3 Positive
5	3	0/3 Positive

Verification of the detection limit of inactivated novel coronavirus

Test Concentration	No. Positive/Total	%Positive
20 $TCID_{50}$/mL	20/20	100

12.3 Cross-reactivity Studies

Cross-reactivity of the SARS-CoV-2 Antigen rapid test kit was evaluated by testing a panel of various microbial that could potentially cross-react for detection of SARS-CoV-2 Antigen. SARS-CoV-2 antigen negative serum were spiked with one of the following cross-reactivity substances to specified concentrations and tested 5 times. Based on the data generated by this study, the microbial tested SARS-CoV-2 Antigen rapid test kit do not cross-react.

Microbial Substance	Test Concentration	Cross-reactivity Results
Escherichia coli	1.0×10^6 CFU/mL	Negative
Hepatitis C Virus (HCV)	1.2×10^5 $TCID_{50}$/mL	Negative
Hepatitis B Virus (HBV)	2.2×10^5 $TCID_{50}$/mL	Negative
Influenza B	$1.0\times10^{6.67}$ $TCID_{50}$/mL	Negative
Influenza A	$1.0\times10^{5.67}TCID_{50}$/mL	Negative
Herpes Simplex Virus-1 (HSV-1)	1.6×10^5 $TCID_{50}$/mL	Negative
Herpes Simplex Virus-2 (HSV-2)	2.1×10^5 $TCID_{50}$/mL	Negative
Human Immunodeficiency Virus – 1 (HIV-1)	3.2×10^5 $TCID_{50}$/mL	Negative
Enterovirus	3.6×10^5 $TCID_{50}$/mL	Negative
Staphylococcus epidermidis	1.0×10^6 CFU/mL	Negative
Legionella pneumophila	3.5×10^6 CFU/mL	Negative
Chlamydia pneumoniae	1.7×10^6 CFU/mL	Negative
Mycoplasma pneumoniae	1.5×10^6 CFU/mL	Negative
Parainluenza virus	1.0×10^5 $TCID_{50}$/mL	Negative
Respiratory syncytial virus	2.1×10^5 $TCID_{50}$/mL	Negative
Adenovirus	1.0×10^5 $TCID_{50}$/mL	Negative
Cytomegalovirus (CMV)	1.0×10^5 $TCID_{50}$/mL	Negative
Epstein-Barr Virus (EBV)	1.0×10^5 $TCID_{50}$/mL	Negative
Varicella Zoster Virus (VZV)	1.0×10^5 $TCID_{50}$/mL	Negative
Parvovirus B19	1.0×10^5 $TCID_{50}$/mL	Negative
Streptococcus pneumoniae	1.0×10^6 CFU/mL	Negative
Streptococcus pyogenes	1.6×10^6 CFU/mL	Negative
Staphylococcus aureus	1.2×10^6 CFU/mL	Negative
Human coronavirus 229E	1.3×10^5 $TCID_{50}$/mL	Negative
Human coronavirus OC43	1.5×10^5 $TCID_{50}$/mL	Negative

12.4 Interference Studies

12.4.1 Endogenous Interference Substances Studies

SARS-CoV-2 antigen weak positive serum and SARS-CoV-2 antigen negative serum were

spiked with one of the following substances to specified concentrations and tested 5 times. Based on the data generated by this study, the endogenous interference substances tested SARS-CoV-2 Antigen rapid test kit do not interfere.

Interfering Substance	Concentration
Bilirubin	0.3mg/mL
Triglyceride	37 mmol/L
Hemoglobin	1 mg/mL
α - interferon	2000 IU/mL
Zanamivir	142ng/mL
Ribavirin	6μg/mL
Oseltamivir	40 μg/mL
Levofloxacin	40 mg/mL
Ceftriaxone	156μg/mL
Meropenem	0.2 mg/mL
Tobramycin	4μg/mL
HAMA	600 ng/mL

12.4.2 Microbial Interference Studies:
Dilute the serum samples of COVID-19 early infection patients that is positive for SARS-CoV-2 Antigen quantitative assay kit (no jaundice, hemolysis and lipids) with negative serum to a positive base sample at the antigen concentration level of 10pg/mL. The microbial samples used in the cross-reaction were added 1:1 to the weak positive serum, and each sample was tested 5 times. The test results of positive samples after adding interfering substances are all positive, indicating no microbial interference was found with the following substances.

Microbial Interfering Substance	Test Concentration	Interference Results
Escherichia coli	1.0×10^6 CFU/mL	Positive
Hepatitis C Virus (HCV)	1.2×10^5 $TCID_{50}$/mL	Positive
Hepatitis B Virus (HBV)	2.2×10^5 $TCID_{50}$/mL	Positive
Influenza B	$1.0\times10^{6.67}$ $TCID_{50}$/mL	Positive
Influenza A	$1.0\times10^{5.67}TCID_{50}$/mL	Positive
Herpes Simplex Virus-1 (HSV-1)	1.6×10^5 $TCID_{50}$/mL	Positive
Herpes Simplex Virus-2 (HSV-2)	2.1×10^5 $TCID_{50}$/mL	Positive
Human Immunodeficiency Virus – 1 (HIV-1)	3.2×10^5 $TCID_{50}$/mL	Positive

Enterovirus	3.6×10^5 $TCID_{50}$/mL	Positive
Staphylococcus epidermidis	1.0×10^6 CFU/mL	Positive
Legionella pneumophila	3.5×10^6 CFU/mL	Positive
Chlamydia pneumoniae	1.7×10^6 CFU/mL	Positive
Mycoplasma pneumoniae	1.5×10^6 CFU/mL	Positive
Parainluenza virus	1.0×10^5 $TCID_{50}$/mL	Positive
Respiratory syncytial virus	2.1×10^5 $TCID_{50}$/mL	Positive
Adenovirus	1.0×10^5 $TCID_{50}$/mL	Positive
HAMA	600 ng/mL	Positive
Cytomegalovirus (CMV)	1.0×10^5 $TCID_{50}$/mL	Positive
Epstein-Barr Virus (EBV)	1.0×10^5 $TCID_{50}$/mL	Positive
Varicella Zoster Virus (VZV)	1.0×10^5 $TCID_{50}$/mL	Positive
Parvovirus B19	1.0×10^5 $TCID_{50}$/mL	Positive
Streptococcus pneumoniae	1.0×10^6 CFU/mL	Positive
Streptococcus pyogenes	1.6×10^6 CFU/mL	Positive
Staphylococcus aureus	1.2×10^6 CFU/mL	Positive
Human coronavirus 229E	1.3×10^5 $TCID_{50}$/mL	Positive
Human coronavirus OC43	1.5×10^5 $TCID_{50}$/mL	Positive

12.5 Hook Effect

12.5.1 Use 200ng/ml recombinant N protein prepared with negative serum for detection, there was no Hook effect detected.

12.5.2 As part of the LoD study the highest concentration of heat-inactivated SARS-CoV-2 stock available ($TCID_{50}$ of 2.0 $x10^4$/mL) was tested. There was no Hook effect detected.

12.6 Clinical Evaluation

Positive Percent Agreement (PPA):

This study adopts a retrospective method, the clinical samples were collected from 62 patients with covid-19 confirmed by PCR in a CDC from January to February 2020. The samples were collected at the same time on the first day of diagnosis. The samples were separated into serum and stored at - 80 °C. A total of 62 samples met the above conditions, and the days of symptoms and the test results were as follows:

No	Days from onset of symptoms	No.antigen positive	No.total number	PPA
1	0～3	27	29	93.10%（95CI：77.23%~99.15%）
2	4～7	33	33	100.00%（95CI：89.42%～100.00%）

Positive Percent Agreement(PPA)= 100%×60/62=96.77%（95CI：（95CI:88.83%~99.61%）

Negative Percent Agreement

Methods: a retrospective study was carried out with 188 samples from local hospital were confirmed negative by PCR.

Negative Percent Agreement (NPA)=100%×186/188=98.94%（95CI：96.21%～99.87%）

13. PROCEDURAL NOTES

13.1. Read this manual carefully before testing the kit.

13.2. It needs to be tested in a laboratory with proper testing conditions. All samples and materials in the testing process shall be handled according to the operation specifications of infectious diseases laboratory.

13.3. Protect the test cassette from moisture.

13.4. All reagents and samples should reach room temperature (18-30℃) before use.

13.5. Do not use lipemic samples.

13.6. Do not use hemolytic samples.

13.7. Do not use turbid contaminated samples.

13.8. Do not store this kit in a frozen condition.

13.9. The interpretation of the test results must be carried out in strict accordance with this manual.

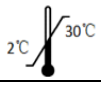
14. DATE OF ISSUE

SARS-CoV-2 Antigen rapid test kit insert.

Version 01, 6th Sep., 2020

15. EXPLANATION OF THE SYMBOLS USED

IVD	In vitro diagnostic medical device
REF	Catalogue Number
LOT	Batch Code
	Manufacturer
	Date of Manufacture

	Use by date
	Do Not Use if Package is Damaged
	Consult Instruction for Use
	Temperature Limit at 2°C~30°C.
	Contents Sufficient for 25 Tests
	Do Not Re-use
	Caution
	Keep Dry

16. GENERAL INFORMATION

Applicant/ Manufacturer

Name: Biohit Healthcare (Hefei) Co., Ltd.

Address: Building D9, Innovation Park, No.800 West Wangjiang Road, High-Tech Zones, Hefei, Anhui Province, P.R. China

Post code: 230088

Contact telephone: +86-551-65652770

Order contact: market@chinabiohit.com

Technical: public@chinabiohit.com

Authorized representative of the European Union

ScheBoÒ · Biotech AG

Netanyastr. 3

35394 Gießen/Germany

Tel.: +49641/4996-0

Fax: +49641/4996-77

e-mail: s.scheefers@schebo.com