SARS-CoV-2 Antigen Quantitative ELISA Assay Kit

(Enzyme-linked Immunoassay)

CV231-K01

Instruction for Use

CE

For research use only
Store at 2℃ -8℃

Distributed in the US and Canada by:

EAGLE BIOSCIENCES, INC.
20A NW BLVD, SUITE 112 NASHUA, NH 03063
P: 617-419-2019 F: 617-419-1110
WWW.EAGLEBIO.COM — INFO@EAGLEBIO.COM

EAGLE
BIOSCIENCES

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, quantitative detection of SARS-CoV-2 N protein is of great clinical significance.

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

2. TEST PRINCIPLE

The kit uses the principle of the double antibody sandwich method to detect SARS-CoV-2 N protein in human serum or plasma. Anti-SARS-CoV-2 N protein antibody was used to prepare microplates in advance. Add the biotin-labelled anti-SARS-CoV-2 N protein antibody and the sample in sequence. If the sample contains N protein, a solid-phase antibody-antigen-biotin-labelled antibody complex is formed. After washing the microplate, streptavidin labelled with HRP is added to further form an immune complex. The unbound substances are washed away, and a substrate solution containing TMB and urea hydrogen peroxide is added to the microplate. The wells are blue in colour, and after being stopped by the stop solution, they can turn into yellow. The absorbance value was read using a microplate EIA reader with a wavelength of 450 nm, using 620nm to 690nm as the reference wavelength. And the content of SARS-CoV-2 N protein in the sample was calculated according to the concentration of the SARS-CoV-2 calibrator.

3. KIT COMPONENTS

No.	Content	Quantity	Reagent
1	Coated microplate	96 wells ×1 plate	Coated with anti-SARS-CoV-2 N protein antibody
2	Conjugate 1	6mL/bottle ×1 bottle	Buffer solution containing biotin-labelled anti-SARS-CoV-2 N protein antibody
3	Conjugate 2	12mL/bottle ×1 bottle	Buffer solution containing horseradish peroxidase-labelled streptavidin
4	Calibrators (CAL1-CAL5)	0.5mL/bottle, 5 bottles in total	Containing different concentrations of recombinant SARS-CoV-2 N protein

5	Control	0.5mL/bottle×1 bottle	Containing recombinant SARS-CoV-2 N protein
6	Substrate solution A	6mL/bottle ×1 bottle	Buffer solution containing peroxide
7	Substrate solution B	6mL/bottle ×1 bottle	Buffer solution containing 3,3',5,5'-tetramethylbenzidine (TMB)
8	Stop solution	6mL/bottle ×1 bottle	Containing sulfuric acid (H_2SO_4)
9	Concentrated wash buffer 20×	30mL/bottle ×1 bottle	Phosphate buffer solution containing surfactants and preservatives
10	Sealing film	3 sheets	/
11	Instructions for use	1 copy	/

4. WARNINGS AND PRECAUTIONS

4.1. Samples for human serum or plasma 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.

4.2. For professional use only.

4.3. This test should be performed at 18 to 30°C. 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. 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.

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

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

4.8. Strong oxidants such as sodium hypochlorite disinfectant may cause the background of TMB substrate to rise, which may lead to misjudgment of the results.

4.9. Do not use expired reagents.

4.10. The components in different batches of kits shall not be mixed.

4.11. The microplate shall be dried to store. The unused microplate shall be immediately put into a clean plastic bag containing desiccant, sealed and moisture-proof. The remaining kits after use shall be put back to 2-8°C for storage in time.

4.12. In order to prevent sample evaporation and pollution, the reaction plate shall be placed in a closed and clean plastic bag or covered with a sealing film during warm cultivation.

4.13. When washing the microplate, the liquid injection volume of each well shall not be

less than 350 µL, and the number of washing the microplate shall not be less than 5 times. Pay attention to check whether the liquid filling head is blocked. After washing the board, dry it on the clean absorbent paper. When washing the board by hand, each washing liquid shall be filled with micropores, and finally it shall be dried on clean water absorbent paper without paper scraps to prevent the accuracy of the test results from being affected.

4.14. The microplate cannot be reused.

5. STORAGE CONDITIONS AND SHELF LIFE

The kit is stored at 2-8°C and the shelf life is 12 months. After opening the coated microplate, add desiccant and seal it in time. The kit is stable for 14 days at 2-8°C.

Liquid reagents such as conjugates, calibrators, substrate, etc. are stable for 60 days before the expire date if stored at 2-8°C after opening. The stop solution and concentrated wash buffer are stable to the indicated expire date after opening.

6. APPLICABLE INSTRUMENTS

Within 15 minutes read the absorbance at 450 nm, using 620nm to 690nm as the reference wavelength if available.

7. SAMPLE REQUIREMENTS

7.1 Applicable to human serum, heparin plasma, EDTA plasma and citrate plasma.

7.2 For serum and heparin plasma, EDTA plasma, or Citrate plasma samples, the samples shall be tested immediately after collection. Serum and heparin plasma, EDTA plasma or Citrate plasma 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 or plasma 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
- Adjustable or fixed range single channel and 8-channel pipettes that can meet the requirements of test sample adding amount
- Test tubes
- Sample collection tubes
- Timer
- Microplate EIA reader
- Incubator: 37 ± 1°C
- Fresh distilled water or deionized water

- Water absorbent paper: strong, non-shedding fiber

9. TEST PROCEDURES

Step 1: Preparation of wash buffer: dilute the concentrated wash buffer 20 times (30mL concentrated wash buffer + 570mL distilled water or deionized water) with fresh prepared distilled water or deionized water in a clean container, mix well for standby, and the diluted wash buffer can be kept at room temperature for 7 days.

Note: Please make sure that there is no crystallization in the concentrated wash buffer before use. If there is crystallization, it can be placed in a water bath pot for heating and dissolution, otherwise the washing effect will be affected.

Step 2: Preparation: take out the kit from the refrigerator, and balance it at room temperature (18-30°C) for 30 minutes. Take out the sample to be tested and let it reach room temperature. Mix the sample well before testing.

Step 3: Design: Take the microplate out of the sealed bag, make one well for the calibrator and one well for the quality control material. Place the microplate on the plate frame according to the designed sample quantity, and mark the plate frame according to the experimental design.

Step 4: Conjugate 1: Add **Conjugate 1** 50 μL to each well.

Step 5:Sample: Add 50μL of **Sample**, control and calibrators respectively：

	1	2	3	4	5
A	CAL1	Sample 3			
B	CAL 2	Sample 4			
C	CAL 3	Sample 5			
D	CAL 4	Sample 6			
E	CAL 5	Sample 7			
F	Control	Sample 8			
G	Sample1	Sample 9			
H	Sample2	Sample 10			

Step 6: Incubation: Stick on the sealing film, shake well with a micro vibrator, incubate at 37 ℃ for 60 minutes.

Step 7: Washing: Wash each well with 350 μL of 20 times diluted wash buffer for 5 times.

Step 8: Conjugate 2: Add **Conjugate 2** 100 μL to each well.

Step 9: Stick on the sealing film, mix with micro vibrator for 5 seconds, and incubate at 37 ℃ for 30 minutes.

Step 10: Washing: Wash each well with 350 μL of 20 times diluted wash buffer for 5 times.

Step 11: Substrate solution: Add 50 μL substrate A to each well, and then add 50μL substrate B to each well.

Step 12: Stick on the sealing film, mix with micro vibrator for 5 seconds, and incubate at 37 ℃ for 15 minutes.

Step 13: Stop solution：Add 50 μL of stop solution into each well, shake gently and mix

well.

Step 14: Reading: Set the wavelength of the Microplate EIA reader at 450nm, using 620nm to 690nm as the reference wavelength, and measure the OD value of each well.

[Sample dilution procedure]

When the SARS-CoV-2 N protein is more than 180.01pg/mL, it should be marked as "＞180.01pg /mL", which can be further tested after diluting the sample. The dilution method is as follows:

- It is recommended to dilute at a dilution ratio of 1:10, for example, 20 μL sample+180 μL normal saline;
- If the diluted sample test is still more than180.01pg/mL, conduct 1:10 dilution again;
- When calculating the sample concentration, it is necessary to input dilution factor to calculate the final sample concentration.

[Result calculation]

- The concentration of the sample to be tested can be calculated as follows：
- Data processing procedure: subtract the absorbance value of Calibrator CAL1 from each test mean, take point 0 as the starting point, the absorbance value of standard solution is y-axis, and the given value of standard solution is X-axis for binomial fitting, and calculate the corresponding concentration value according to the absorbance value of sample and control after subtracting.

10. INTERPRETATION OF THE RESULTS

10.1. Cut-off value

According to the test results of clinical samples, the Cut-off value is determined as 2.97pg/mL.

10.2. Quality control standard

(1) Check the OD value of calibrator (CAL 5). When CAL 5 ≤1.000, the test is invalid, and the test shall be repeated.

(2) Check the OD value of the calibrator (Calibrator CAL 1). When CAL1>0.100, the test is invalid and should be retested.

(3) The test results of quality control products should be within ± 20% of the target value, otherwise the experiment is invalid, and the experiment should be repeated.

10.3. The samples with the test concentration ≥2.97 pg/mL are judged as SARS-CoV-2 N protein positive samples.

10.4. When the test concentration is >180.01pg/mL, it can be reported as ">180.01pg/mL"; if it is necessary to further determine the sample concentration, it can refer to the method in the sample dilution procedure to test the sample after dilution.

10.5. 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.

10.6. SARS-CoV-2 N protein novel coronavirus nucleocapsid protein, N protein 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 protein cannot completely exclude the infection of SARS-CoV-2. The reason is that when the content of SARS-CoV-2 N protein in the sample is below the conventional detection limit, or anti-N protein antibody has been produced in the serum, SARS-CoV-2 N protein cannot be detected.

10.7. When the specific antibody appears in the blood, SARS-CoV-2 N protein is neutralized, which leads to the decrease of N protein content in the serum to the degree that it can not be detected.

10.8. 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.

10.9. Each laboratory can establish its own reference range according to the actual situation.

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.

11.3. The presence of sodium azide in the sample will affect the experimental results. Sodium azide cannot be used as a sample preservative.

11.4. If the microplate is not washed sufficiently or there is residual liquid, it may cause unreliable results.

11.5. If the time interval of sample adding is too long, it may cause the deviation of test results.

12. PERFORMANCE CHARACTERISTICS

Blank limit

LOB: Using the blank solution as a sample, repeat the test 20 times, and the LOB is not greater than 1.08 pg/mL.

Linear range

In the concentration range of 2.89-180.01pg/mL, the correlation coefficient (r) is not less than 0.9900.

Accuracy

Recovery test is used to evaluate the accuracy of the kit. The recovery rate should be in the range of 85.0% ~ 115.0%.

Precision

Three serum and EDTA plasma samples were used for the intra-assay variation study. Each sample was tested 10 times and 3 batches were tested. Each sample got 10 analysis results per batch. The concentration of serum ranges from 6.49 pg/mL to 168.02 pg/mL, and CV ranges from 4.80% to 9.01%. The concentration range of EDTA plasma was 5.88 pg/mL to 159.35 pg/mL, and the range of CV was 3.53% to 9.35%.

Three serum and EDTA plasma samples were used for the inter-assay variation study. Each sample was tested 10 times, and 3 batches were tested. Each sample was analyzed 30 times. The concentration of serum ranges from 6.49 pg/mL to 168.02 pg/mL, and the CV ranges from 5.80% to 9.23%. The concentration range of EDTA plasma was 5.88 pg/mL to

159.35 pg/mL, and the range of CV was 5.65% to 10.07%.

Cross-reactivity

Cross-reactivity of the SARS-CoV-2 Antigen quantitative assay kit was evaluated by using clinical serum samples listed below, which come from patients infected with different pathogen. Five samples were tested with three lots of SARS-CoV-2 Antigen quantitative assay kit. None of the antigen in the listed underlying conditions cross-reacted with SARS-CoV-2 antigen quantitative assay kit by generating false positive results.

Condition	Sample Testing Times	Results
Q fever Rickettsia	5	Negative
Chlamydia pneumoniae	5	Negative
Mycoplasma pneumoniae	5	Negative
Parainluenza virus	5	Negative
Respiratory syncytial virus	5	Negative
Influenza A	5	Negative
Legionella pneumophila	5	Negative
Adenovirus	5	Negative
Influenza B	5	Negative
HAMA	5	Negative

Endogenous/exogenous interference

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 in multiple replicates. No false positivity or false negativity results were found with the following:

Number	Interfering substance	Concentration
1	Bilirubin	0.3mg/mL
2	Triglyceride	37 mmol/L
3	Hemoglobin	10mg/mL
4	α - interferon	2ng/mL
5	Zanamivir	142ng/mL
6	Ribavirin	6μg/mL
7	Oseltamivir	1μg/mL
8	Levofloxacin	2 mg/mL
9	Ceftriaxone	156μg/mL
10	Meropenem	0.2 mg/mL

11	Tobramycin	4μg/mL

Clinical Evaluation

Positive Percent Agreement (PPA):

Novel coronavirus pneumonia (COVID-19) patients with serum samples were detected in 101 cases, of which 32 patients had a course of disease less than 3 days, 38 patients had a 4~7 days course, 31 patients had a 8~14 days course of disease, and antigen tests were performed on the samples, and the results were statistically analyzed.

No	Days from onset of symptoms	Antigen positive	Total number	PPA
1	≤3	30	32	93.75%（95CI：79.19%~99.23%）
2	4～7	38	38	100.00%（95CI：90.75%~100.00%）
3	8～14	28	31	90.32%（95CI：74.25%~97.96%）
4	≤14	96	101	95.05%（95CI：88.82%~98.37%）

Sample Source: PCR confirmed COVID-19 patients serum sample

Positive Percent Agreement(PPA)= 96/101=95.05%（95CI：88.82%~98.37%）

Negative Percent Agreement

Methods: a retrospective study was carried out with 649 samples from local hospital, including 246 samples of other respiratory tract infections (The serum samples were collected in the Clinical Laboratory of the local hospital from February to July 2020, the patients were confirmed negative by PCR), 85 samples were pregnancy test samples (The serum samples were collected in the Clinical Laboratory of the local hospital from February to May 2020, the patients were confirmed negative by PCR), 77 abnormal samples of rheumatoid factor (The serum samples were collected in the Clinical Laboratory of the local hospital from May 2020, the patients were confirmed negative by PCR), 155 physical examination serum samples (The samples were collected in the Clinical Laboratory of the local hospital on June 2020, the patients were confirmed negative by PCR)，86 plasma samples of inpatients in other departments (The samples were collected in the Clinical Laboratory of the local hospital on June 2020, the patients were confirmed negative by PCR)

Group no.	Sample types	No. of samples	No. of positive samples
1	Other respiratory infection samples	246	0
2	Pregnancy test samples	85	0
3	Rheumatoid factor samples	77	0
4	Physical examination serum samples	155	0

5	Plasma samples of inpatients in other departments	86	0
6	Total	649	0

Negative Percent Agreement (NPA)=100%×649/649=100.00% （95CI：99.43%,100.00%）

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. The operation of this kit requires a certain degree of professionalism, and the operators shall receive professional training.

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. False negative results will be caused when the antigen titer in the sample is lower than the minimum detection limit.

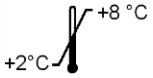
14. DATE OF ISSUE

SARS-CoV-2 Antigen quantitative assay kit insert.

Version 02, 6th July, 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~8°C.
	Contents sufficient for 96 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

Name: Salofa Oy

Address: Örninkatu 15, 24100 Salo, Finland

Tel: +358 44 554 4702

Email: info@salofa.com

Authorized representative in the Americas

Name: Eagle Biosciences, Inc.

Address: 10 Columbia Drive, Amherst, NH

Tel: +1-617-419-2019

Email: info@eaglebio.com