SARS-CoV-2 IgG/Neutralizing Antibody Rapid Test Kit (Colloidal Gold)

Instructions for Use (IFU)

【PRODUCT NAME】

SARS-CoV-2 IgG/Neutralizing Antibody Rapid Test Kit (Colloidal Gold)

【PRODUCT CODE】

G10402

【PACKAGE AND SPECIFICATION】

20Tests/box (1Test ×20) 、40 Tests /box (1Test ×40)

【INTENDED USE】

For in vitro qualitative detect of human IgG antibodies against SARS-CoV-2 and neutralizing antibodies that block the interaction between the receptor binding domain of the viral spike glycoprotein (RBD) with the ACE2 cell surface receptor in serum, plasma and whole blood. This test is only provided for use by clinical laboratories or to healthcare workers for point-of-care testing. Home-testing by lay persons are subject to local legislations.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped non-segmented positive-sense RNA virus. It is the cause of coronavirus disease 2019 (COVID-19), which is contagious in humans. SARS-CoV-2 has several structural proteins including spike (S), envelope (E), membrane (M) and nucleocapsid (N). The spike protein (S) contains a receptor binding domain (RBD), which is responsible for recognizing the cell surface receptor, angiotensin converting enzyme-2 (ACE2). It is found that the RBD of the SARS-CoV-2 S protein strongly interacts with the human ACE2 receptor leading to endocytosis into the host cells of the deep lung and viral replication.

Infection with the SARS-CoV-2 initiates an immune response, which includes the production of antibodies in the blood. The secreted antibodies provide protection against future infections from viruses, because they remain in the circulatory system for months to years after infection and will bind quickly and strongly to the pathogen to block cellular infiltration and replication. These antibodies are named neutralizing antibodies.

For in vitro diagnostic use only. For professional use only

【TEST PRINCIPLE】

For the detect of human IgG antibodies against SARS-CoV-2, the Kit use immunocapture method.

For the detect of neutralizing antibodies, this Kit is a blocking rapid detection tool, which mimics the virus neutralization process. The protein-protein interaction between RBD and hACE2 can be blocked by neutralizing antibodies against SARS-CoV-2 RBD.

Key components: the recombinant novel coronavirus antigen (RBD and nucleocapsid protein) and chicken IgY labeled by colloidal gold are as tracers, the nitro cellulose membrane was coated with mouse anti-human IgG antibody, human ACE2 receptor protein (hACE2) and goat anti-chicken IgY antibody.

When specimens are processed and added to the test device, neutralizing antibodies present in the specimen will bind to the RBD labeled colloidal gold and block the protein-protein interaction between RBD and hACE2. The unbound RBD labeled colloidal gold as well as any RBD labeled colloidal gold bound to non-neutralizing antibody will be captured on the test line (2). The color density of neutralizing antibodies test line is inversely proportional to RBD-NAbs present within the sample. Thus, an absent or faint test line indicates high levels of RBD-NAbs, whereas a dark or strong test line suggests lack of RBD-NAbs in the specimen. Human IgG antibodies against SARS-CoV-2 will combine with colloidal gold labeled novel coronavirus antigen to form a complex, which is captured by the mouse anti-human IgG antibody coated on the test line (1) form a colored band. The colloidal gold labeled chicken IgY antibody is bound to the goat anti- chicken IgY antibody coated on the control line (C), which acts as a quality control line. The test line (2) will get weaker as the increase concentration of the neutralizing antibodies and disappear at a high concentration of the neutralizing antibodies.

【COMPONENT】

Parts	20Tests/Kit	40Tests/Kit	Main components
Test Kit	20Tests/Kit (1Test×20)	40Tests/Kit (1Test ×40)	The detection line were coated with mouse anti-human IgG antibody, human ACE2 receptor protein (hACE2), the quality control lines were coated with goat anti-chicken antibody, the recombinant novel coronavirus antigen (RBD and nucleocapsid protein) and chicken IgY labeled by colloidal gold are as tracers.
Desiccant	20 pouchs	40 pouchs	Silica Gel
Sample Diluent	20 bottles	40 bottles	Solution of trimethylaminomethane hydrochloride (0.02M Tris-HCl)
Safety Lancet	20 pcs	40 pcs	/
Dropper	20 pcs	40 pcs	/
Alcohol Pad	20 pcs	40 pcs	/
Instruction for Use	1 sheet	1 sheet	/

Materials Required But Not Supplied in the Kit: Timer, Gloves.

【STORAGE AND STABILITY】

1. Store at 4~30℃ in the sealed pouch up to the expiration date, and the validity is tentatively 24 months. Do not freeze.
2. The test cassette should be used within 1 hour after taking out from the aluminum foil bag.
3. Keep away from sunlight, moisture and heat.

【WARNING AND PRECAUTIONS

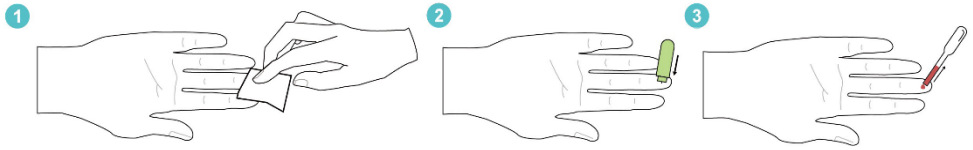
1. Test cassettes are single use only. Do not reuse test cassettes.
2. For IN VITRO Diagnostic use only.
3. This test is only intended for detecting the presence of IgG antibodies and neutralizing antibodies against SARS-CoV2, not for any other viruses or pathogens.
4. All human blood products should be handled as potentially infectious material.
5. Never pipette by mouth or allow reagents or patient sample to come into contact with skin.
6. Optimal results will be obtained by strict adherence to this protocol. Reagents must be added carefully to maintain precision and accuracy.
7. Performing the assay outside the prescribed time and temperature ranges may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
8. The components in this kit have been quality control tested as a master lot unit. Do not mix components from different lot numbers. Do not mix with components from other manufacturers.
9. Care should be exercised to protect the reagents in this kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results. Do not heat-inactivate samples.
10. Keep storage boxes dry.
11. Do not use test cassettes if foil pouch is punctured or damaged.
12. Testing materials should be disposed of in accordance with local, state and/or federal regulations.
13. Do not use after expiration date.
14. Please read the instructions carefully before operation and follow the instructions.
15. Please use fresh samples as much as possible, and avoid using samples contaminated with bacteria, hemolysis, jaundice, or excessive blood lipid.
16. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
17. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air-conditioning.

【SPECIMEN COLLECTION AND PREPARATION】

1. The recommended samples for this kit are serum, plasma and whole blood. Plasma and whole blood can be collected by blood collection tube or centrifuge tube with EDTA-2K or heparin sodium anticoagulant.
2. The samples collected with the correct medical technology should be returned to room temperature before testing. Jaundice, hemolysis, lipemia, and cloudy samples cannot be used. Severe hemolytic or heat-inactivated specimens are not recommended.
3. Samples should be tested as soon as possible. If the test cannot be completed within 8 hours, the samples can be stored at low temperature. Serum and plasma can be stored for 7 days at 2-8℃or for 6 months at -20℃, and whole blood can be stored for 3 days at 2-8℃. Do not freeze and thaw samples repeatedly.
4. Bring the test kit and specimen to room temperature before start.

【FINGERTIP BLOOD SPECIMEN COLLECTION】

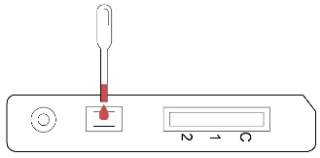
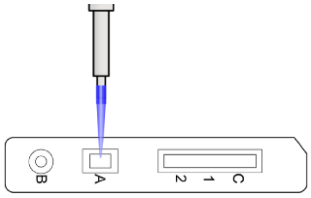
1. Wipe to clean the puncture site on your finger with the alcohol pad.
2. Remove the cap from safety lancet, push the lancet firmly against the puncture site.
3. Use the disposable pipette to draw the blood from puncture site.



【TEST METHOD】

Note: Ensure test kits and specimen are equilibrated to room temperature (20-25°C) before starting the test.

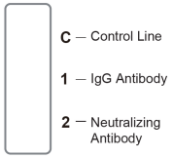
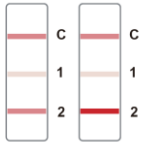
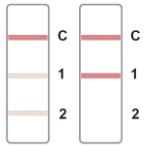
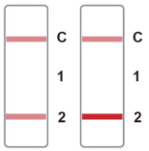
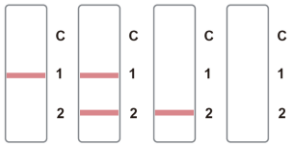
1. Tear off the foil pouch just prior to use, take out the test strip/cassette and place the test cassette on a clean and level surface.
2. Add specimen to the sample well on the test cassette:

Fingertip Blood Specimen	**Serum, Plasma or Whole Blood Specimen**
Refer to the previous section "FINGERTIP BLOOD SPECIMEN COLLECTION", collect fingertip blood with safety lancet, add **one (1) drop (20μL)** of blood with disposable pipette into the sample well on the test cassette. 	Use a disposable pipette, transfer **10μL** serum (or plasma), or **20μL** whole blood into the sample well on the test cassette. 

3. Open the buffer tube by twisting off the top. Hold the buffer bottle vertically and 1 cm above the buffer well. Add **three (3) drops (100 μL)** of the buffer into buffer well on the test cassette.

4. Start the timer. Read the test results between **25 and 30 minutes**. Do not read the results after 30 minutes.

【INTERPRETATION OF TEST RESULTS】

The result reading window on test cassette has three regions: the control line region (C), the IgG test line region (1), and the neutralizing antibody test line region (2). When control line appears, a colored line in IgG test line region (1) indicates the presence of IgG antibodies; the color density in neutralizing antibody test line region (2) is in an **inverse correlation** with the neutralizing antibody level. The test results should be interpreted following the instructions below.	
IgG positive and neutralizing antibody negative: Three lines appear. One colored line appears in the control line region (C), a colored line appears in IgG test line (1) region, and a colored line appears in neutralizing antibody test line (2) region which is ***darker or equal than C line***.	 IgG Positive and Neutralizing Antibody Negative
IgG positive and neutralizing antibody positive: One colored line appears in the control line region (C), a colored line appears in IgG test line (1) region, and the color in neutralizing antibody test line (2) region is ***lighter than C line***. When the neutralizing antibody test line (2) region does not show a visible line, it represents a high neutralizing antibody level.	 IgG Positive and Neutralizing Antibody Positive
NEGATIVE: Two lines appear. One colored line should be in the control line region (C), and a colored line appears in neutralizing antibody test line (2) region which is ***darker or equal than C line***.	 Negative
INVALID: Control line fails to appear. Insufficient buffer volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the procedure with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.	 Invalid

- **Result determination time:** The result should be judged within ***25~30 minutes*** after the sample is added into the sample loading well, and the result displayed after ***30 minutes*** is invalid.

【LIMITATIONS OF TEST METHOD】

1. This product is only suitable for qualitative test and auxiliary diagnosis.
2. The test results are only for clinical reference and should not be the only basis for clinical diagnosis and treatment. The clinical management of patients should be considered in combination with their symptom, physical signs, medical history, other laboratory tests, therapeutic reaction, and epidemiological information.
3. The hemolytic, lipemia, jaundice, and contaminated samples may affect the test results. Such samples should be avoided.
4. We do not test all types of collection tubes that may be used for this kit; therefore, for blood sample collection tubes from different manufacturers, different results may be obtained due to different raw materials and additives. Each laboratory shall make its own judgment on the suitability of the blood collection tubes.
5. The test is for in vitro diagnostic use only.
6. This test is only provided for use by clinical laboratories or to healthcare workers for point-of-care testing. Home-testing by lay persons are subject to local legislations.
7. The test is for qualitative detection of SARS-CoV-2 IgG antibody and neutralizing antibodies in human fingerstick blood, serum and plasma specimens. It does not indicate the quantity of the antibodies. The intensity of the test line does not necessarily correlate to SARS-CoV-2 antibody titer in the specimen.
8. The test results should be interpreted between 25 and 30 minutes after addition of buffer. The test results should not be interpreted after 30 minutes.
9. Negative results do not preclude SARS-CoV-2 infection and immunity to SARS-COV-2 infection, it should not be used as the sole basis for patient management decisions. False positive results for antibodies may occur due to cross-reactivity from preexisting antibodies or other possible causes.
10. A negative result can occur if the quantity of antibodies for the SARS-CoV-2 virus present in the specimen is below the detection limit of the assay, or if the virus has undergone minor amino acid mutation(s) in the epitope recognized by the antibody used in the test.
11. Results from antibody testing should not be used to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
12. A positive result may not indicate previous SARS-CoV-2 infection. Consider other information, including clinical history and local disease prevalence, in assessing the need for a second but different serology test to confirm an immune response.
13. Not for the screening of donated blood.

【PERFORMANCE CHARACTERISTICS】

1. Clinical Study

The clinical sensitivity of the kit is determined by specimens collected from 93 participants who received Pfizer-BioNTech COVID-19 mRNA vaccine (Tozinameran or BNT162b2) at the Centro Diagnostico Delta S.r.l. located in Piazza San Giuseppe Moscati, 8 - 82030 Apollosa (Benevento) Italy between January 2021and March 2021. The clinical specificity of the kit is determined by specimens collected from 317 uninfected and unvaccinated participants from Heilongjiang Province Hospital in China. The reference reagent used in the clinical study is cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit manufactured by GenScript USA Inc. The kit showed 92.47% of sensitivity and 99.68% of specificity.

Table 1. Overall Clinical Study Results

Reagent test results	Reference Reagent cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit		Subtotal
	positive	negative	
positive	86	1	87
negative	7	316	323
Subtotal	93	317	410

Positive Percent Agreement (PPA)= 86/93(92.47%) (95%CI: 85.1%-96.9%)

Negative Percent Agreement: (NPA) = 316/317 (99.68%) (95%CI: 98.2%-100.0%)

Accuracy= (86+316)/410×100%= 98.05%

Kappa= 2×27169/284034= 0.94＞0.5

2. Assay Cross-Reactivity

Cross-reactivity of the Kit was evaluated using serum or plasma samples (collected before August 2019) which contain antibodies to the pathogens listed below. No false positivity was found with the following:

Table 2: Cross-reactivity Results

Potential cross-reactant			
Potential cross-reactants	No. of samples	Potential cross-reactants	No. of samples
Anti-Flu A (IgM)	10	Human coronavirus panel (IgM)	10
Anti-Flu B (IgM)	10	EB Virus antibody (IgM)	10
anti-HKU1 (beta coronavirus)	10	HIV-1 and HIV-2 (IgM)	10
anti-OC43 (beta coronavirus)	10	Adenovirus (IgM)	10
anti-NL63 (alpha coronavirus)	10	Human Metapneumovirus (hMPV) (IgM)	10
anti-229E (alpha coronavirus)	10	Parainfluenza virus 1-4 (IgM)	10
anti-rhinovirus (IgM)	10	Enterovirus (IgM)	10
anti-HCV (IgM)	10	Rhinovirus (IgM)	10
anti-HBV (IgM)	10	Streptococcus pneumoniae (IgM)	10
ANA	10	Mycobacterium tuberculosis (IgM)	10
anti-respiratory syncytial virus (IgM)	10	Mycoplasma pneumoniae (IgM)	10
anti-Haemophilus influenzae. (IgM)	10		

3. Potentially Endogenous Interfering Substances: Low titer COVID-19 antibody positive serum samples and COVID-19 antibody negative serum samples were spiked with one of the following substances to specified concentrations and tested in multiple replicates. No false positivity or false negativity was found with the following:

Table 3: Potentially Endogenous Interfering Substances

Bilirubin Conjugated	0.3 mg/mL	Antibody (HAMA) Human Serum Albumin	50 mg/mL
Hemoglobin	8 mg/mL	Levofloxacin	200 mg/L
Human Anti-mouse	780 ng/mL	α-IFN	200 mg/L
Bilirubin Unconjugated	0.4 mg/mL	Abidol	50 mg/L
Triglycerides	15 mg/mL	Tobramycin	10 mg/L
Cholesterol	5 mg/mL	Ribavirin	40 mg/L
Rheumatoid Factor	2000 IU/mL	Ceftriaxone	420 mg/L
Histamine hydrochloride	4 mg/L	Meropenem	210 mg/L

Oseltamivir carboxylate	1 mg/L	Human IgM	0.5 mg/mL
Zanamivir	1 mg/L	Human IgG	9 mg/mL

【EXPLANATION OF LABELS】

	In Vitro Diagnostic Use		See Instruction for Use		Catalog #
	Batch Number		Expiry Date		Manufacturing Date
	Do not reuse		Store between 4～30ºC		Keep away from Sunlight
	Keep Dry		Manufacturer		EU Authorized Representative
	CE Mark				

【BASIC INFORMATION】

JOYSBIO(Tianjin) Biotechnology Co., Ltd.

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【DATE OF APPROVALAND AMENDMENT OF IFU】

March-2021