SARS-CoV-2 Neutralization Antibody Screening ELISA Kit

Catalog No. BSKV0004(96 wells)
For use with Serum and cell culture supernatants.

For Research Use Only. Not for use in diagnostic procedures.

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INTRODUCTIONS

Coronaviruses are enveloped viruses with a positive-sense RNA genome and with a nucleocapsid of helical symmetry. 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. The subpopulation of the binding antibodies that can block cellular

infiltration and replication of the virus are named neutralizing antibodies. This kit applies to the screening of the SARS-CoV-2 neutralization antibody.

PRINCIPLE OF THE ASSAY

This assay employs a competitive ELISA format and is uniquely suitable for rapid high-throughput screening of the Anti-SARS-CoV-2 neutralizing antibody in serum or cell culture supernatants. The ACE2 receptor protein has been pre-coated onto a microplate. Controls or samples are pipetted into the wells, and then a horseradish peroxidase-conjugated recombinant SARS-CoV-2 RBD fragment (RBD-HRP) is added to the wells together. The protein-protein interaction between RBD-HRP and ACE2 can be blocked by neutralizing antibodies against SARS-CoV-2 RBD. Following incubation and wash steps, a substrate is added. A colored product is formed in proportion to the amount of SARS-CoV-2 Neutralization Antibody present in the sample. The reaction is terminated by the addition of acid, and absorbance is measured at 450nm. A Positive control is prepared from SARS-CoV-2 Neutralization Antibody (Mouse IgG) to ensure the results' validity.

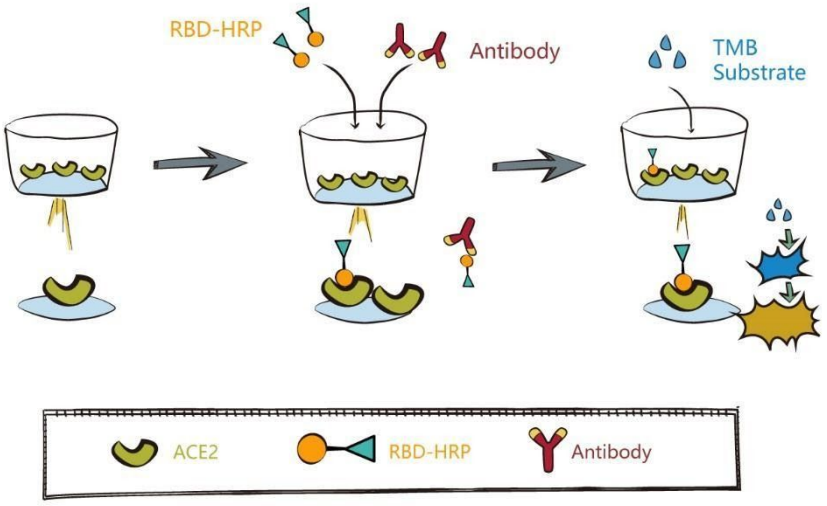

Figure 1. Schematic diagram of the assay

MATERIALS SUPPLIED

Table 1. Kit Components

Kit Components	**96 wells Quantity/Size**
Aluminum pouches with a Microwell Plate coated with ACE2 receptor protein (8·12)	1 plate
Positive control	2 vials
Negative control	2 vials
Recombinant SARS-CoV-2 RBD fragment conjugated to horseradish peroxidase (HRP)	2 vials
Diluent Buffer	1 bottle
Wash Buffer Concentrate 20x (PBS with 1% Tween-20)	1 bottle
Substrate Solution	1 bottle
Stop Solution	1 bottle
Adhesive Films	4 pieces
Package insert	

STORAGE

Table 2. Storage of the kit

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8°C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="7">Opened/ Reconstituted Reagents</td><td>Negative control</td><td rowspan="5">May be stored for up to 1 month at 2 - 8°C.**</td></tr>
<tr><td>Diluent Buffer</td></tr>
<tr><td>Wash Buffer Concentrate 20x</td></tr>
<tr><td>Substrate Solution</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Positive control
Recombinant SARS-CoV-2 RBD fragment conjugated to horseradish peroxidase (HRP)</td><td rowspan="2">Reconstituted reagents shall not be reused.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2 - 8°C.**</td></tr>
</table>

**Provided and this is within the expiration date of the kit.

MATERIALS NEEDED BUT NOT SUPPLIED

1. Microplate reader (450nm).
2. Micropipette and tips: 0.5-10, 2-20, 20-200, 200-1000μL.
3. Microplate shaker.
4. Double-distilled water or deionized water.
5. Coordinate paper.
6. Graduated cylinder.

PRECAUTIONS FOR USE

1. Store kit reagents between 2℃and 8℃. After use, all reagents should be immediately returned to cold storage (2℃ to 8℃).
2. Please perform simple centrifugation to collect the liquid before use.
3. To avoid cross-contamination, please use disposable pipette tips.
4. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material. Avoid contact of skin or mucous membranes with kit reagents or specimens. In the case of contact with skin or eyes, wash immediately with water.
5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate, and substrate reagent. Mix all reagents and samples well before use.
6. After washing, the microtiter plate should be fully pat dried. Do not use absorbent paper directly into the

enzyme reaction wells.

7. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond the expiration date on the label.
8. Each sample, controls, blank and optional control samples should be assayed in duplicate or triplicate.
9. Adequate mixing is essential for good results. Use a microplate shaker at the lowest frequency or Shake by hand at 5 min intervals when there is no vortexer.
10. Avoid microtiter plates drying during the operation.
11. Any variation in diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.
12. This method can effectively eliminate the interference of the soluble receptors, binding proteins, and other factors in biological samples.

SAMPLE COLLECTION AND STORAGE

1. **Serum** - Collect whole blood in untreated test tubes or, for example, an anticoagulant-free tube such as BD Vacutainer Serum tubes. Incubate undisturbed at room temperature for 20 min. Centrifuge at 3,000 rpm for 10 min at 4°C. Immediately aliquot supernatant (serum) and store samples at -80°C. Minimize freeze/thaw cycles.
2. **Cell Culture Supernates** - Pipette cell culture media into a centrifuge tube and centrifuge at 1,500 rpm for 10 min at 4°C. Immediately aliquot supernatant and store samples at -80°C. Minimize freeze/thaw cycles.
3. Dilute samples at the appropriate multiple (recommended to do a pre-test to determine the dilution factor).

REAGENT PREPARATION

1. Bring all reagents to room temperature before use.
2. **Wash Buffer** - Dilute 10mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature, and mix gently until the crystals have completely dissolved.
3. **Positive control** –Reconstitute the Positive control with 1mL of Diluent Buffer. Allow the Positive control to sit for a minimum of 15 minutes with gentle agitation before making dilutions.
4. **Sample**- Dilute Serum sample with Diluent Buffer with a volume ratio of **1:20**. For example, dilute 5 µL of the sample with 95 µL of Diluent Buffer. For the Cell Culture Supernatants sample, it is recommended to do a pre-test to determine the dilution factor.
5. **Working solution of RBD-HRP**: Reconstitute the RBD-HRP with 40 µL of Diluent Buffer to be RBD-HRP Stock Solution. Make a 1:100 dilution of the RBD-HRP Stock Solution with the Diluent Buffer in a clean plastic tube.

   The working solution should be used within one day after dilution.

GENERAL ELISA PROTOCOL

1. Prepare all reagents and working controls as directed in the previous sections.
2. Determine the number of microwell strips required to test the desired number of samples plus the appropriate number of wells needed for running blanks and controls. Remove extra microwell strips from the holder and store them in a foil bag with the desiccant provided at 2-8℃ sealed tightly.

3. Add 50 µL of positive control, negative control, or sample per well, then add 50 µL of the working solution of RBD-HRP to each well. Cover with the adhesive strip provided and incubate for 30 minutes at RT. Adequate mixing is essential for good results. Use a microplate shaker at the lowest frequency. Avoid placing the plate in direct light.
4. Aspirate each well and wash, repeating the process three times for a total of five washes. Wash by filling each well with Wash Buffer (350 µL) using a squirt bottle, manifold dispenser, or auto-washer. Complete removal of the liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of Substrate Solution to each well. Incubate for 5-20 minutes at RT. Avoid placing the plate in direct light.
6. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. (optionally 630nm as the reference wavelength;610-650nm is acceptable).

ASSAY PROCEDURE SUMMARY

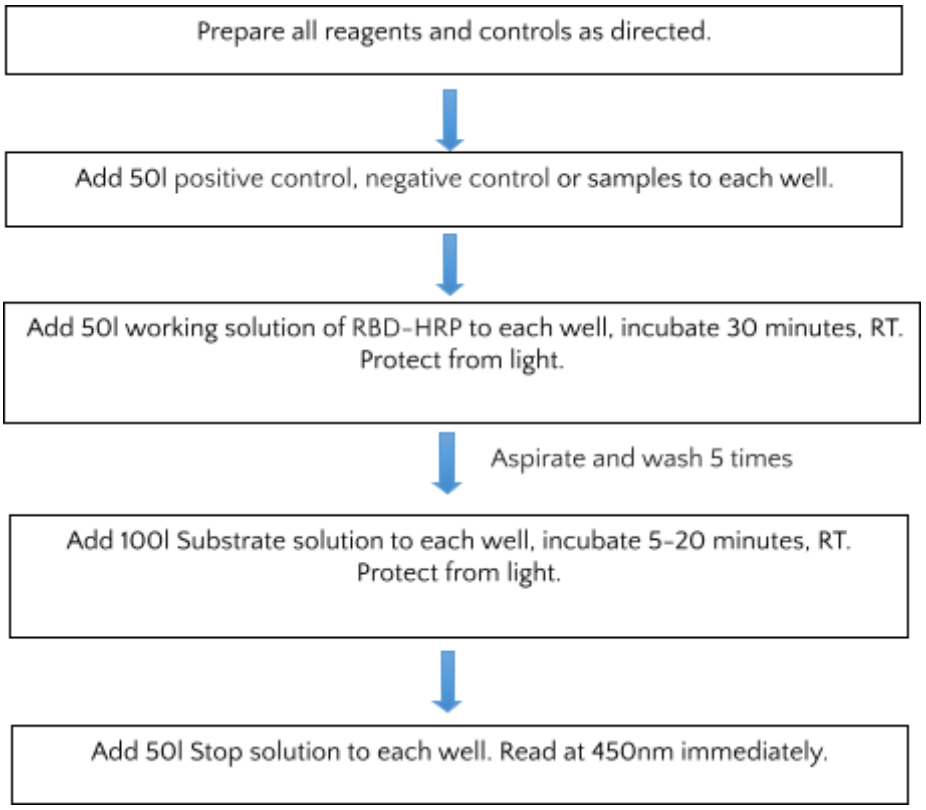

Figure 2. Assay procedure summary

TECHNICAL HINTS

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. To avoid cross-contamination, change pipette tips between additions of each control, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.

5. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

CALCULATION OF RESULTS

1. Average the duplicate readings for each s positive control, negative control, and sample.
2. Each assay must include both Positive and Negative Controls to assure the validity of the results.
3. According to the test data, the 30% inhibition rate can be used for the cutoff of the SARS-CoV-2 Neutralization Antibody Screening ELISA Kit. The experimenter can determine the result of the sample by comparing the inhibition rate to the following table.

   Inhibition Rate = (1 - OD value of Sample / OD value of Negative Control) × 100%

Table 3. Test Result Interpretation

Inhibition Rate *	Result	Test Result Interpretation
≥ 30%	Positive	SARS-CoV-2 Neutralization Antibody positive
< 30%	Negative	No detection of SARS-CoV-2 Neutralization Antibody

*The cutoff value is based on the validation of Sars-CoV-2 positive samples and negative samples in the Bioss laboratory. It is suggested that each laboratory should establish its own reference range according to the actual situation. When using the kit to evaluate the effectiveness of immunization (vaccine), it is recommended to collect serum samples before and after immunization respectively for comparison so as to evaluate whether neutralizing antibodies are produced.

PERFORMANCE CHARACTERISTICS

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

SPECIFICITY: This assay is specific to SARS-CoV-2 neutralizing antibodies.

METHOD VERIFICATION:

The positive serum was tested by diluted in serial two-fold steps in Diluent Buffer to evaluate the SARS-CoV-2 Neutralization Antibody Screening ELISA Kit's sensitivity. The figure below shows the results with the SARS-CoV-2 Neutralization Antibody Screening ELISA Kit for tested samples:

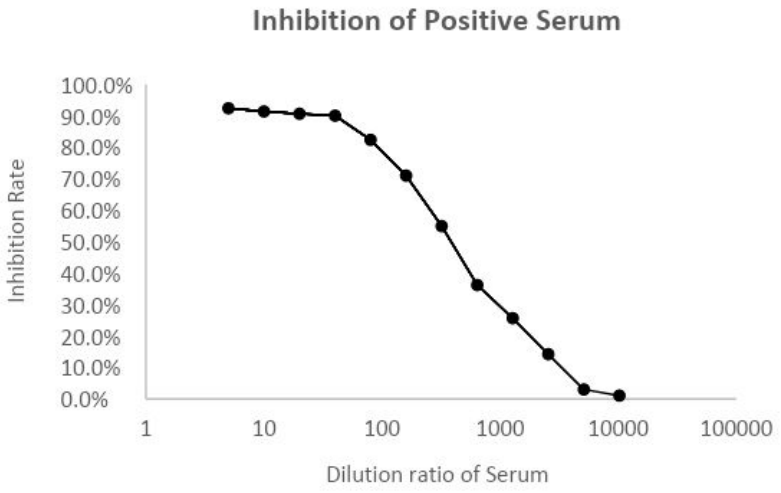

Figure 3. Test Result of positive serum

In order to validate the performance of the SARS-CoV-2 Neutralization Antibody Screening ELISA Kit, the positive and negative controls and 80 samples (20 Positive Samples and 60 Negative Samples) were tested in duplicate. The table below summarizes the results with the SARS-CoV-2 Neutralization Antibody Screening ELISA Kit tested samples:

Table 4. Test Result of Positive Sample

Samples Name	Optical Density	Inhibition Rate	Result Reported
1# Positive Sample	0.707	60.34%	Positive
2# Positive Sample	0.200	88.70%	Positive
3# Positive Sample	0.542	69.32%	Positive
4# Positive Sample	0.593	66.40%	Positive
5# Positive Sample	0.234	86.74%	Positive
6# Positive Sample	0.778	55.95%	Positive
7# Positive Sample	0.952	46.06%	Positive
8# Positive Sample	0.452	74.39%	Positive
9# Positive Sample	0.394	77.71%	Positive
10# Positive Sample	0.456	74.19%	Positive
11-20# Positive Sample	1.021-0.113	42.15%-93.62%	Positive

Table 5. Test Result of Negative Sample

Samples Name	Optical Density	Inhibition Rate	Result Reported
1# Negative Sample	1.526	13.52%	Negative
2# Negative Sample	1.856	-5.14%	Negative
3# Negative Sample	1.805	-2.25%	Negative
4# Negative Sample	1.830	-3.70%	Negative
5# Negative Sample	1.774	-0.50%	Negative
6# Negative Sample	1.889	-7.05%	Negative
7# Negative Sample	2.016	-14.21%	Negative
8# Negative Sample	1.967	-11.47%	Negative
9# Negative Sample	1.853	-4.99%	Negative
10# Negative Sample	1.631	7.58%	Negative
11-60# Negative Sample	2.105--1.318	-19.24%--25.32%	Negative