SARS-CoV-2
Neutralization Antibody Detection ELISA Kit

Catalog No. TBS3240

User Manual

TribioScience, Inc.

365 San Aleso Ave.
Sunnyvale, CA 94085
Tel: 408-498-0197, Fax: 650-618-5498
Email: info@tribioscience.com

1. ASSAY PRINCIPLE

SARS-CoV-2 Neutralization Antibody Detection ELISA Kit is based on the competitive principle. The supplied microtiter plate has been pre-coated with recombinant SARS-CoV-2 Spike RBD-human Fc protein. When HRP conjugated recombinant human ACE2 protein is added simultaneously to wells along with diluted human serum/plasma samples, immobilized RBD will bind human ACE2 protein. Binding of RBD to human ACE2 protein is dose-dependently inhibited by presence of anti-SARS-CoV-2 human neutralization antibodies in serum samples. Following the sample incubation step, unbound ACE2-HRP conjugate is washed away. TMB substrate is then added, and reacts with the HRP enzyme, resulting in blue color development. An acidic stop solution is then added to terminate color development reaction. The absorbance value of the well is measured at a wavelength of 450 nm on a spectrophotometric plate reader. A Positive Control is a known human antibody IgG1 which is specific for SARS-CoV-2 spike protein with a binding epitope for RBD binding site. The positive control antibody is validated by an established Pseudovirus infection of cultured human cells for proven neutralization function. A human IgG1 isotype control is used as a Negative Control sample. The absorbance of unknown sample can be compared to the Positive and Negative controls to determine absence or presence of anti-SARS-CoV-2 human neutralization antibodies. In the competition assay, the greater the amount of anti-SARS-CoV-2 human neutralization antibodies in the sample, the lower the color development and optical density reading.

2. KIT COMPONENTS

Component	Quantity	Volume	Part No.
RBD-human Fc Pre-coated 96-well Strip Plate	1 plate	N/A	TBS3240-01
Anti-SARS-COV-2 Human IgG1 Positive Control	1 vial	50 µL	TBS3240-02
Human IgG1 Negative Control	1 vial	25 µL	TBS3240-03
Assay Buffer	2 bottles	25 mL	TBS3240-04
Wash Buffer (20x)	1 bottle	25 mL	TBS3240-05
HRP-Conjugated Human ACE2 Protein	1 vial	25 µL	TBS3240-06
TMB Substrate	1 bottle	12 mL	TBS3240-07
Stop Solution	1 bottle	6 mL	TBS3240-08
Instruction Manual	1 booklet	N/A	NA

3. KIT STORAGE

Upon receipt, store all kit components at 2-8°C. Once opened, it is recommended that the kit should be used within 1 month. Unused Strip wells can be stored at 2-8°C for 1 month in the sealed aluminum pouch containing desiccant to minimize exposure to moisture. Do not use the kit beyond its expiration date.

4. MATERIALS NEEDED BUT NOT SUPPLIED

- Microplate reader with OD450 nm wavelength filter
- An orbital microplate shaker
- High-precision pipette/multichannel pipette and pipette tips
- Polypropylene microfuge tubes
- Deionized or distilled water
- Absorbent paper towels

5. SAMPLE COLLECTION

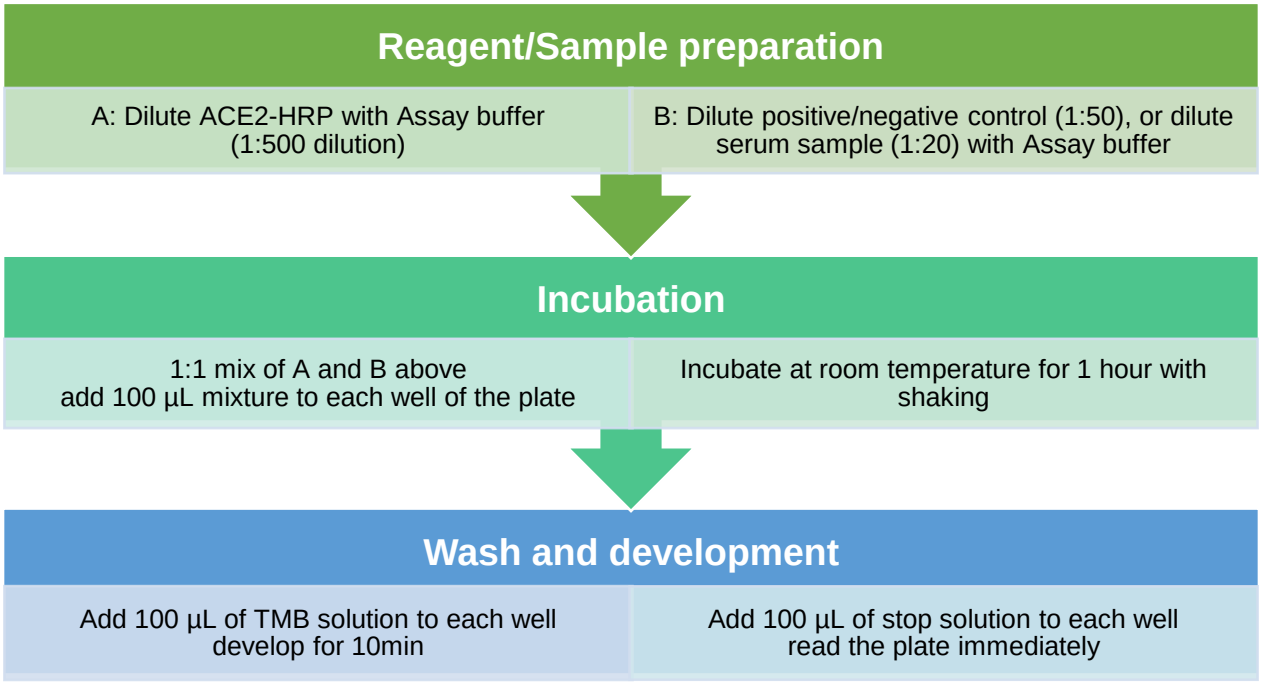
This assay is recommended for use with human serum or plasma. Use with other sample types such as SARS-CoV-2 immunized animal serum is possible but has not been validated. The sample collection protocols below is provided for user reference.

5.1. Platelet-Poor Plasma: Collect plasma using Heparin, EDTA, or Citrate as an anticoagulant. Centrifuge samples for 15 minutes at 1,000x*g* at 4°C within 30 minutes of collection. It is recommended that samples should be centrifuged for 10 minutes at 10,000x*g* for complete platelet removal. Collect the supernatant for testing.

5.2. Serum: Use a serum separator tube and allow samples to clot for at least 1 hours at room temperature or overnight at 2-8°C before centrifugation for 20 minutes at 1000x*g*. Collect the supernatant for testing.

5.3. Sample storage: it is recommended that samples should be used immediately upon preparation. For long-term storage sample aliquots should be prepared and stored at -20°C if used within 1 month, or -80°C if used within 6 months. Long term storage can result in protein degradation and denaturation, which may result in inaccurate results. Avoid repeated freeze/thaw cycles for all samples.

6. ASSAY PROTOCOL



6.1. Reagent preparation

6.1.1. Equilibrate plate to room temperature before opening the sealed pouch. Warm kit reagents to room temperature prior to use.

6.1.2. 1 x Wash Buffer: Prepare 500 mL of Working Wash Buffer by diluting the supplied 25 mL of 20x Wash Buffer with 475 mL of deionized or distilled water. Wash Buffer can be stored at 2-8°C once prepared. No preservatives should be added to the Wash Buffer.

Note: If crystals have formed in wash buffer, warm to room temperature and mix it gently until crystals have completely dissolved.

6.1.3. ACE2-HRP conjugate: centrifuge the tube to bring down the liquid prior to open the tube. Prepare 6 mL of ACE2-HRP conjugate working solution by adding 12 µL of the ACE2-HRP conjugate concentrate in 6 mL of Assay Buffer. Diluted ACE2-HRP conjugate leftover should be discarded after use.

6.1.4. Positive and Negative Control samples: centrifuge the tubes to bring down the liquids prior to opening the vials. Pipet 10µL of the Positive Control (or Negative Control) and add it to 490 µL of Assay Buffer to make the working solution.

6.2. Sample preparation

Serum or plasma samples should be brought to room temperature before performing the assay. It is recommended that human serum and plasma samples to be diluted 20-fold prior to testing. Add 10 µL of serum or plasma to 190 µL Assay Buffer to prepare diluted testing samples. For other sample types, customer is expected to perform validation experiments to justify the utility according to the applications.

6.3. Standard preparation (optional for quantification)

This is a semi-quantitative assay. If quantitative determination is needed, a full standard curve using the positive control should be performed.

The following are instructions for the preparation of a standard dilution series which will be used to generate the standard curve used in quantitative testing. The standard curve is used to determine the concentration of anti-SARS-COV-2 neutralization antibodies in unknown samples (see the Calculation of Results section). The following will prepare sufficient volumes to run the standard dilution series in duplicate. Prepared standard dilutions should be used immediately and not stored for future use.

The Positive Control/Standard contains Human anti-SARS-CoV-2 Neutralization Antibody at 100 µg/mL. To prepare a standard serial dilution:

a. Briefly spin down the Positive Control vial
b. Pipet 25 µL of the Positive control and add to a microfuge tube containing 475 µL Assay buffer. The concentration of this top standard is 5.0 µg/mL.
c. Pipet 150 µL of the 5.0 µg/mL solution and add to a second microfuge tube containing 300 µL of Assay Buffer. This is a 3-fold dilution of the top standard.
d. Repeat Step C to prepare remaining tubes of serial 3-fold dilutions, resulting in 1666.7 ng/mL, 555.6 ng/mL, 185.2 ng/mL, 61.7 ng/mL, 20.6 ng/mL, 6.9 ng/mL, 2.3ng/mL.
e. Use Assay Buffer as the 0 ng/mL standard.

6.4. Assay procedures

Prepare all reagents, working standards, and samples as described in the previous sections of this manual.

6.4.1. Mix 60 µL of ACE2-HRP conjugate working solution (prepared in step 6.1.3) with 60 µL of 1:20 diluted serum or plasma samples (prepared in step 6.2), Positive Control, and Negative control (prepared in step 6.1.4).

6.4.2. Add 100 µL of mixture from step 6.4.1 to appropriate wells.

6.4.3. Cover the plate with an adhesive plate sealer and incubate for 1 hour at room temperature with gentle shaking on a microtiter plate shaker.
6.4.4. Aspirate the liquid from each well and wash 4 times. Wash by adding approximately 200-300 µL of 1x Wash Buffer using a multi-channel pipette, manifold dispenser, or automated washer. Allow each wash to sit for 1-2 minutes before completely aspirating. After the last wash, aspirate to remove any remaining 1x Wash Buffer then invert the plate and tap against clean absorbent paper towel.
6.4.5. Add 100 µL of TMB substrate solution to each well. Gently tap the plate to mix. Cover the plate from direct light exposure. Allow the plate to sit for 5-10 minutes at room temperature. Monitor blue color development.
6.4.6. Add 100 µL of Stop Solution to each well. The blue color turns to yellow.
6.4.7. Read the plate immediately on an ELISA plate reader at OD450 nm wavelength.

7. ASSAY SPECIFICATIONS

7.1. Target: Human antibodies that block RBD binding to human ACE2 receptors.
7.2. Specificity: This kit is for the detection of Human anti-SARS-CoV-2 neutralization antibodies.
7.3. Sample Types: This kit is recommended for use with human serum/plasma. Use with other sample types like SARS-CoV-2 immunized animal serum is possible but has not been validated.
7.4. Sensitivity: typically, about 7.0 ng/mL with IC50 at approximately 50.0 ng/mL.
7.5. Performance: Intra-Assay CV < 10.0%; Inter-Assay CV < 15.0%.
7.6. Limitations: This kit is for Research Use Only.

8. CALCULATION OF RESULTS

Calculate the mean absorbance for each set of duplicate controls, and samples and subtract the blank optical density. For quantitative assays, use appropriate software that can perform four or five - parameter logistic regression models for most accurate curve-fitting and sample concentration determination.

OD450nm reading for controls provided in the kit:

Sample	OD450 reading
Negative control	> 1.5
Positive control	< 0.5

Data interpretation:

The data is calculated as the % of inhibition:
% of Inhibition = (1-OD450 value of serum sample/OD450 value of negative control) x 100%

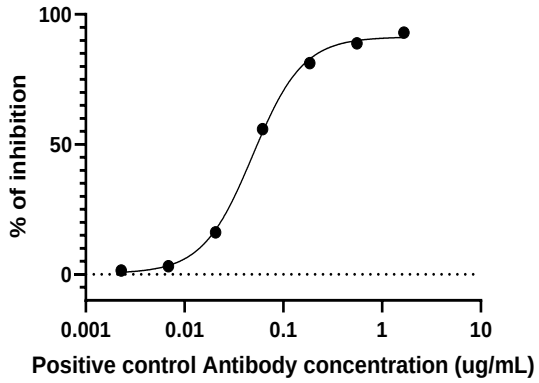
Suggested cut off:

Based on the serum sample tested from recovered patient and normal human, we recommended to use 40.0% as the cut off.

Item	% of inhibition	Result indication
ELISA testing result	> or = to 40.0%	Presence of SARS-CoV-2 neutralizing antibody
	< 40.0%	Absence of SARS-CoV-2 neutralizing antibody

Typical Data

The following standard curve is an example only and should not be used to calculate results for tested samples. A new standard curve must be generated for each set of samples tested.



Typical Data

Serum samples from SARS-CoV-2 recovered patients (n=10) and healthy individuals (n=10) were tested using Tribioscience SARS-CoV-2 neutralization antibody detection ELISA kit. The serum samples from recovered patients (>90%) have detectable level of neutralization antibody (average % of inhibition is about 61%). In contrast, the serum samples from healthy individuals do not have any obvious presence of neutralization antibody (average % of inhibition is 27%).