# SARS-CoV-2 Total Antibody Detection ELISA Kit

## (Targeting RBD of Spike Proteins)

**Catalog No. BSKV0005(96 wells)**

**For use with Serum.**

***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. This kit is configured and optimized to support research for SARS-CoV-2; it can be used to detect the RBD antibodies in the serum of patients with SARS-CoV-2 infection as early as possible and serving as a tool to evaluate the immune level of other animals to the SARS-CoV-2 RBD.

## PRINCIPLE OF THE ASSAY

This assay employs a quantitative sandwich enzyme immunoassay technique and is uniquely suitable for rapid high-throughput detecting the levels of the SARS-CoV-2 total antibody in serum. The recombinant SARS-CoV-2 RBD fragment has been pre-coated onto a microplate. Controls or samples are pipetted into the wells, and then a horseradish peroxidase-conjugated RBD fragment (RBD-HRP) is added to the wells, producing an antigen-antibody-antigen "sandwich complex." Following incubation and wash steps, a substrate is added. A colored product is formed in proportion to the amount of SARS-CoV-2 total antibody present in the sample. The addition of acid terminates the reaction, and absorbance is measured at 450nm. A Positive control is prepared from SARS-CoV-2 RBD antibodies to assure the validity of the results.

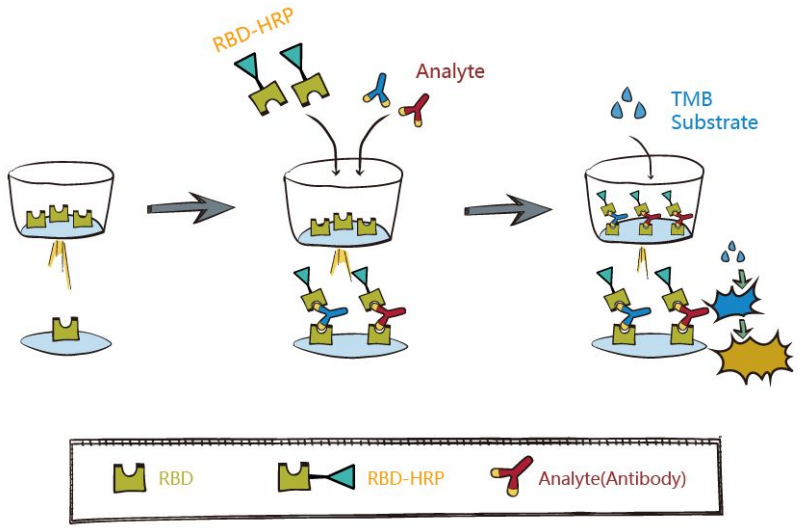


Figure 1. Schematic diagram of the assay

## MATERIALS SUPPLIED

*Table 1. Kit Components*

| **Kit Components** | **96 wells Quantity/Size** |
|---|---|
| Aluminum pouches with a Microwell Plate coated with SARS-CoV-2 RBD fragment (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</td><td rowspan="2">Reconstituted reagents shall not be reused.</td></tr>
<tr><td>Recombinant SARS-CoV-2 RBD fragment conjugated to horseradish peroxidase (HRP)</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. 37℃ Incubator.
4. Double-distilled water or deionized water.
5. 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.
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. 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 300μL 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.
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.
6. ***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. Incubate for 60 minutes at 37℃.
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 (350μL) 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

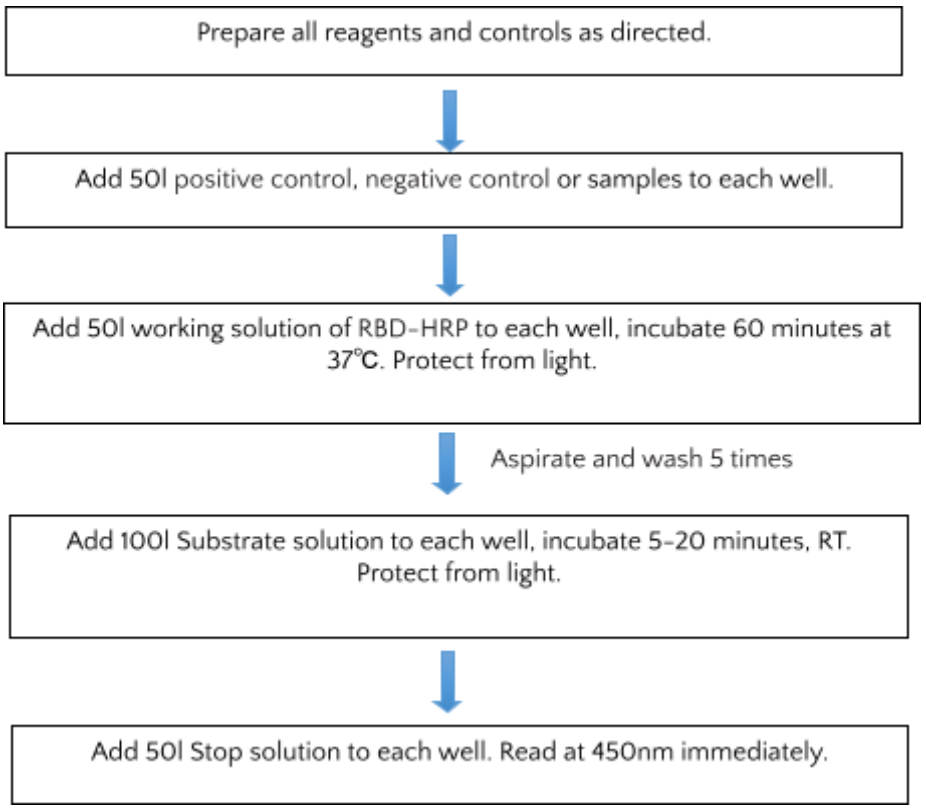


Figure 2. Assay procedure summary

## 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 positive control, negative control, and sample.

2. To assure the validity of the results, each assay must include both Positive and Negative Controls. If the average OD450 value of each control does not meet the requirements (below), the test should be

repeated.

*Table 3 Laboratory quality control requirements:*

| Control | Requirement | Instruction |
|---|---|---|
| Positive Control | OD450≥ 1.0 | If the controls do not meet the requirements, the test should be repeated. |
| Negative Control | OD450< 0.3 | |

3. According to the test data, the operator can determine the result of the sample by comparing the ratio of **P/N** to the following table.

*Table 4 Interpretation of results*

| Items* | Value Results** | Result | Test Result Interpretation |
|---|---|---|---|
| P/N | ≥ 2.5 | Positive | SARS-CoV-2 Total Antibody positive |
| | < 2.5 | Negative | No detection of SARS-CoV-2 Antibody |

***P: OD450 value of Sample

N: OD450 value of Negative Controls

****The cutoff value is based on the validation of SARS-CoV-2 positive serum and negative serum in the Bioss laboratory. It is suggested that each laboratory should establish its own reference range according to the actual situation. When using this 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 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 RBD total antibodies.

**METHOD VERIFICATION:**

To evaluate the sensitivity of the SARS-CoV-2 Total Antibody Detection ELISA Kit, positive serum and negative serum were tested by diluted in serial two-fold steps in Diluent Buffer. The figure(below) shows the results with the SARS-CoV-2 Total Antibody Detection ELISA Kit for tested samples:

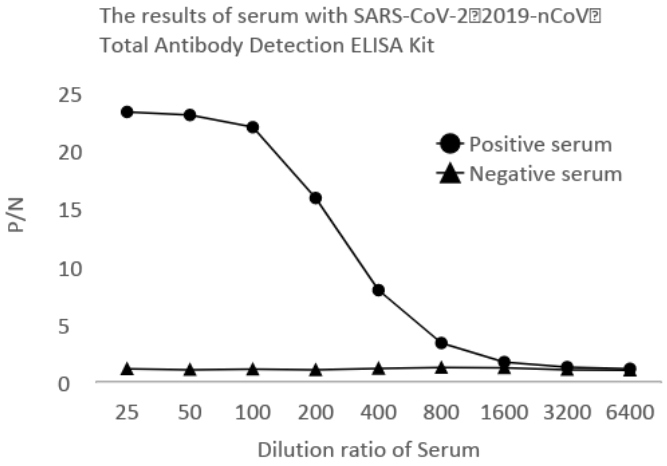


Figure 3. Test results of serum