SARS-CoV-2 RBD+M Protein Human IgG ELISA Kit

Catalog# EA200002

This ELISA kit is for research use only and it is not intended for diagnostic use.

PRODUCT INFORMATION

This kit is used for the detection of COVID-19 human IgG antibodies in human serum, plasma or cell culture supernatant in vitro. This assay is validated manually, but can be adapted to an automated instrument.

GENERAL DESCRIPTION

COVID-19 is a symptom after contracting SARS-CoV-2. SARS-CoV-2 is a single strand RNA coronavirus. It is composed of several proteins including Spike (S) protein, Envelope (E) protein, membrane (M) protein, and nucleocapsid (N). Research data show that the RBD region of the spike protein could bind to ACE2 receptor on the human cell membrane and use it as a mechanism for cell entry. IgG is the most abundant immunoglobulin to be found and produced in human body in response to an invader. Specific IgG is produced 7-14 days after contracting SARS-CoV-2.

PRINCIPLE OF THE ASSAY

Microtitration wells coated with alternating strips of recombinant fusion protein containing SARS-CoV-2 Spike receptor binding domain and a piece of virion surface domain of the membrane (RBD+M) viral antigen and negative control antigen are exposed to test specimens, which may contain human IgG antibodies directed against SARS-CoV-2 RBD+M protein. After incubation period, unbound antibodies in the test sample are washed away. Bound SARS-CoV-2 RBD+M protein IgG antibodies are then incubated with an anti-human/mouse IgG peroxidase conjugate. Following a second wash cycle, the bound peroxidase conjugate is detected by the addition of ABTS peroxidase substrate. The assay is measured spectrophotometrically to indicate the abundance of SARS-CoV-2 RBD+M protein human IgG antibodies present in a sample.

STORAGE AND STABILITY

1. This product can be shipped globally at room temperature. Store all reagents at 2-8°C upon arrival and when not used. The expiration date printed on the box label indicates the limit of product stability.

2. The foil packs containing ELISA strips should be warmed to room temperature (20-25°C) before opening to prevent condensation. Once opened, microtitration strips may be stored at 2-8°C until expiration date, provided that desiccated conditions are maintained.

KIT CONTENT

Product	Catalogue	Per Kit
SARS-Cov-2 RBD + M Protein ELISA Plate (Alternating + and - Strips)	SP866C	1 plate
Positive Control (Anti-RBD chimeric monoclonal antibody)	SPC866	0.5 mL
Negative Control	595-200	0.5 mL
Sample Diluent	595-413	30 mL
Peroxidase Conjugate	595-001	12 mL
Wash Buffer Concentrate 20X	82710	60 mL
ABTS Peroxidase Substrate	595-419	12 mL
Stop Solution	595-417	10 mL

Sufficient reagents are supplied to run 48 tests.
NOTE: Kit plate and conjugate lots are matched and must be used as a set. All reagents are ready-to-use EXCEPT for the wash concentrate, which is supplied as a 20X concentrate.

INSTRUCTIONS FOR USE

Reagent Preparation
Prepare the following reagents and samples before beginning the assay procedure. All reagents and samples should be prepared at room temperature (20-25 °C) prior to start of the assay and may remain at room temperature during testing. Store reagents at 2-8°C immediately after use.

Sample Diluent – 1 vial (30 ml)

Contains normal goat and bovine serum in phosphate buffered saline and Proclin as a preservative. The sample diluent is stable for a minimum of 1 year when stored at 2-8°C.

Positive Control - 1 vial (0.5 ml)

The vial contains ready-to-use positive control at the dilution needed for the test. No further dilutions are required. The positive control is stable for a minimum of 1 year when stored at 2-8°C.

Negative Control - 1 vial (0.5 ml)

The vial contains ready-to-use negative control at the dilution needed for the test. No further dilutions are required. The negative control is stable for a minimum of 1 year when stored at 2-8°C.

Wash Concentrate (20X) - 1 vial (60 ml)

The wash concentrate contains tris buffer with surfactant. Check the wash concentrate for presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Wash solution is stable for 3 weeks from the date of preparation if stored at 2-8°C. Dilute wash concentrate as needed.

Dilute wash concentrate at 1:20 with deionized or distilled water in a clean glass or plastic screw cap container (e.g. add 50 ml of wash concentrate to 950 ml of water). Mix gently by inverting several times to avoid excessive foaming. To ensure sufficient volume when using an automated plate washer, 1x60 ml of wash concentrate is provided to allow for excess wash solution to prime the plate washer (1200 ml total wash solution after dilution).

Peroxidase Conjugate - 1 vial (12 ml)

The vial contains ready-to-use conjugate. One vial provides enough conjugate for 12 strips (1 plate). If more than 1 vial is need, pool the content of both vials in a clean glass or plastic screw cap container and mix gently by inverting several times to avoid excessive foaming. Opened conjugate is stable for 30 days. Label and date the vial and store at 2-8°C.

ABTS Peroxidase Substrate - 1 vial (12 ml)

Each vial contains 12 ml of 2,2' Azino-di[3-ethyl-benzthiazoline-sulfonate] solution. The vial contains ready-for-use substrate. ABTS is stable for a minimum of 1 year when stored at 2-8°C.

Stop Solution - 1 vial (5 ml)

The stop solution contains 1.25% sodium fluoride.
CAUTION: Avoid contact with eyes and skin. If contact is made, wash affected area with copious amounts of water and seek medical attention immediately.

SPECIMEN COLLECTION AND PREPARATION

A. A. Obtain blood and allow clot to form. Insoluble materials should be removed by centrifugation. Remove the serum aseptically. Serum samples should be refrigerated as soon as possible after collection. If not assayed within 48 hours, samples should be aliquoted and frozen. Avoid repeated freezing/thawing. Samples should not contain sodium azide.

B. Dilute serum at 1:50 in sample diluent (e.g. add 5 ul of serum sample to 245 ul of 1X sample diluent). If not assayed immediately, diluted samples should be stored at -20°C or below.

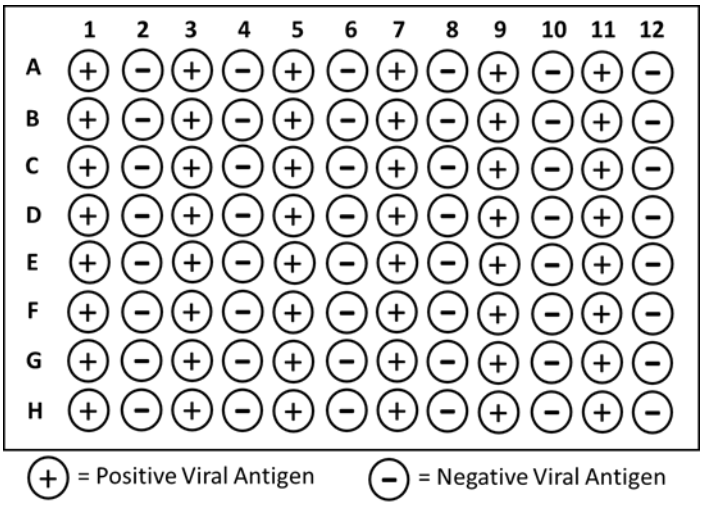
Procedural Notes

1. Review complete instructions before performing the test.

2. Strips of ELISA plate are removable. Remove unused strips and store as described under "Storage and Stability". Before testing begins, the user should inspect ELISA strip holders and ensure all strips are secure. A white stabilizer residue is normally observed on the bottom of unused wells.

Strip holders should be handled with care to ensure that no strip is dislodged during testing. It is recommended that each strip is numbered with a laboratory marker prior to use. Additionally, since strips are pre-coated with positive viral antigen and negative control antigen (alternating 6 positive and 6 negative antigen-coated strips/strip holder), it is recommended that each strip is labeled with a "+" or "-" to indicate the type of antigen coat in each well.

NOTE: Assembled strip holders always start with a positive antigen (+) coated strip. Subsequent strips alternate between negative control (-) antigen and positive (+) antigen, so that strips 1,3,5,7,9, and 11 are pre-coated with positive (+) antigen and strips 2,4,6,8, 10, and 12 are pre-coated with negative (-) control antigen. A schematic representation of this is shown below.



Avoid touching bottom surface of wells, as this may affect readings. ELISA strips must only be used once. Strip holders may be used again.

3. Dispose of all used materials as biohazard waste.

4. A new pipette tip must be used for each sample. Never touch the pipette tip to the bottom of the well. If plastic troughs are used, ensure that they have a dedicated purpose (do not use the same trough for peroxidase conjugate and ABTS peroxidase substrate).

WASH PROCEDURE

Proper washing and aspiration of strip wells are required to obtain accurate and reliable results. Wells should be washed 5 times after both sample incubation and conjugate incubation.

1. Aspirate wells into a waste flask.
2. Fill each well with wash solution.
3. Aspirate wells.
4. Repeat steps 2 and 3 for an additional 4 cycles (total of 5 washes).
5. Invert plate and tap firmly on absorbent paper to remove excess liquid. Be careful not to dislodge strips.

TEST PROCEDURE

All samples and controls should be tested on both positive viral antigen and the negative control antigen wells. Use the enclosed record sheet to identify the location of each serum and type of strip (+ or - antigen) used in the test.

1. In a small dilution tube, make a 1:50 dilution of the test serum in 1X sample diluent and mix well (e.g. add 5 ul of serum to 245 ul of 1X sample diluent).

2. Fit the strip holder with required number of pre-coated positive viral antigen and negative control viral antigen strips. Mark the appropriate strips with a (+) or (-). Allow one well to be used for the negative control sera and one well for the positive control sera.

3. Pipette 100 ul of each diluted serum sample, the negative control and positive control into the appropriate (+) and (-) marked wells.

4. Cover the wells and incubate at 37°C for 45±1 minutes.
5. After incubation, wash each well five (5) times with 1X wash solution (refer to the Wash Procedure).
6. Pipette 100 ul of liquid ready-to-use peroxidase conjugate to each test well. Cover the wells and incubate at 37°C for 45±1 minutes.
7. After incubation, wash each well five (5) times with 1X wash solution (refer to Wash Procedure).
8. Pipette 100 ul of liquid ready-to-use ABTS peroxidase substrate into each test well.
9. Incubate the plate at room temperature (20-25°C) for 30 minutes.
10. Blank the micro reader on air and read the absorbance of the colorimetric reaction in each well at 405nm.
11. If the plate is not read immediately, pipette 25 ul of stop solution into each test well. Read the plate at 405 nm within 15 minutes.

INTERPRETATION OF RESULTS

1. **As the SARS-CoV-2 Spike Receptor Binding Domain and Membrane Protein Human IgG ELISA Assay is strictly for research use only, it is recommended that each laboratory establish its own criteria for performance of these research reagents.**
2. In our testing for quality control, we use the following criteria,
    a. Negative Control - after subtracting the absorbance in the negative control antigen well, it should produce a net absorbance on the positive viral antigen of ≤ 0.250 at 405 nm.
    b. Positive Control - after subtracting the absorbance in the negative control antigen well, it should produce a net absorbance on the positive viral antigen of ≥ 0.600 at 405 nm.
    c. A sample may be considered positive by the following criteria - determine the difference (Δ) between the sample absorbance at 405 nm on the positive viral antigen well and the absorbance at 405 nm on the negative control antigen well. **The difference (Δ) should be greater than or equal to 0.300 for a sample to be considered positive.**

    Example #1: Positive Sample
    Given a sample absorbance of 1.101 at 405 nm on the positive viral antigen well and a sample absorbance of 0.190 at 405 nm on the negative control antigen well.
    The difference (Δ) between the above absorbance is 0.911.
    This difference is greater than or equal to 0.300. This sample is considered positive.

    Example #2: Negative Sample
    Given a sample absorbance of 0.347 at 405 nm on the positive viral antigen well and a sample absorbance of 0.319 at 405 nm on the negative control antigen well.
    The difference (Δ) between the above absorbance is 0.028.
    This difference is less than 0.300. This sample is considered negative.
3. If the test specimen falls between the absorbance range of 0.25 to 0.4 nm after subtracting the absorbance of the negative control antigen well, this is considered an equivocal result and additional/alternative testing should also be performed on the test specimen by the end user to verify the results.

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