Technical Manual

COVID-19 Human IgA PharmaGenie ELISA Kit

- Catalogue Code: SBRS1720
- Indirect Principle
- Research Use Only

Contents

Introduction

The Novel Coronavirus (SARS-CoV-2) S1 RBD protein Human IgA ELISA Kit is an in vitro indirect ELISA for the quantitative measurement of human IgA antibody against SARSCoV-2 S1 RBD protein in human serum. This ELISA kit is for research use only, not for therapeutic or diagnostic applications.

Description and Principle

The Assay Genie Sandwich ELISA kit is a highly sensitive assay for the Quantitative / Semi-Quantitative measurement of a specific analyte in the following samples: serum, blood, plasma, cell culture supernatant and other related supernatants and tissues.

How do our ELISA kits work?

This COVID19 human IgA antibody ELISA kit employs an indirect ELISA method. In this kit, standard 96-well plates (12 strips with 8 wells/strip) are coated with the SARS-CoV-2 S1 RBD protein, which combines with the corresponding antibody present in a sample and Positive Control, which used as calibration curve for interpretation purposes. The wells are washed, and biotinylated anti-human IgA antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells, and color develops in proportion to the amount of COVID19 S1 RBD protein human IgA antibody bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. The same procedure is conducted on another standard 96-well plate coated with human Albumin protein, which is used for background subtraction purposes.

The Positive Control is from an inactivated serum sample which contains SARS-COV-2 S1 RBD protein human IgA antibody. We do not know the exact amount of SARS-COV-2 S1 RBD protein human IgA antibody in the Positive Control sample. The Positive Control can be used as a calibration curve for interpretation purposes in different assays.

Storage & Expiry

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see table below.

Kit Contents

Each kit contains reagents for 96 assays including:

No.	Component	96-Well Kit	Storage
1	Microplate coated with SARS-CoV-2 S1 RBD protein	8 x 12	1 month at 4°C*
2	96 wells (12 strips x 8 wells) coated with Albumin protein	8 x 12	1 month at 4°C*
3	Wash Buffer Concentrate (20X)	40ml	1 month at 4°C*
4	Positive Control (contains SARS-Cov-2 S1 RBD protein human IgA antibody)	2 vials	1 week at -80°C
5	Biotinylated Anti-Human IgA	2 vials	1 week at 4°C
6	HRP-Streptavidin concentrate	1 vial	Do not store and reuse
7	TMB One-Step Substrate Reagent	24ml	1 month at 4°C
8	Stop Solution	16 ml of 0.2 M sulfuric acid	N/A
9	Assay Diluent B	15 ml	1 month at 4°C
10	5X Sample Diluent (5X)	25ml	1 month at 4°C

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

Additional materials required:

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.

Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use.

2. 5X Sample Diluent should be diluted 5-fold with deionized or distilled water before use to make 1X Sample Diluent.

3. 5X Assay Diluent B should be diluted 5-fold with deionized or distilled water before use to make 1X Assay Diluent B.

4. Dilute sample (human serum) with 1X Sample Diluent (Item J) 500 times. For example, add 1 µl serum + 499 µl 1X Sample Diluent. Mix the diluted sample well and evenly for the best results.

Note 1: The user needs to calculate the amount of the sample used for the whole test. Please reserve sufficient amount of sample in advance.

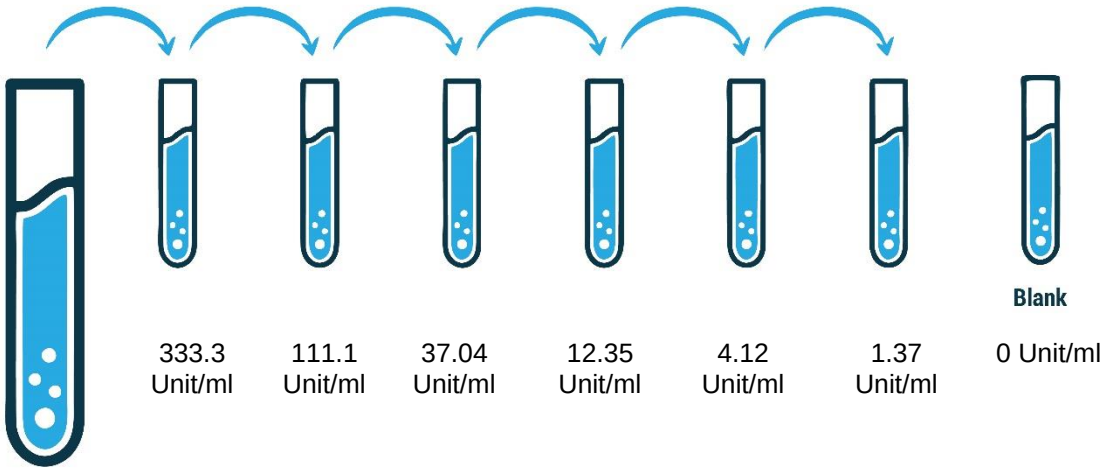
Note 2: Avoid using samples with severe hemolysis, precipitate, contamination by bacteria or protein suspension.

Note 3: The use of EDTA, heparin sulfate, sodium citrate, or other anticoagulants will not affect the results.

5 . Preparation of Positive Control calibration curve: Briefly spin the vials of Positive Control. Add 400 µl 1X Sample Diluent into each Positive Control vial to prepare a 1000 Unit/ml Positive Control solution and mix thoroughly. Pipette 320 µl 1X Sample Diluent into 2 sets each of 7 tubes. Use the 1000 Unit/ml Positive Control solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1X Sample Diluent serves as the zero (0 Unit/ml).

DILUTION SERIES

Positive Control + 400 µl | 160µl | 160µl | 160µl | 160µl | 160µl | 160µl



1000 Unit/ml | 333.3 Unit/ml | 111.1 Unit/ml | 37.04 Unit/ml | 12.35 Unit/ml | 4.12 Unit/ml | 1.37 Unit/ml | 0 Unit/ml

6. If the Wash Concentrate (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 40 ml of Wash Buffer Concentrate into deionized or distilled water to yield 800 ml of 1X Wash Buffer.

7. Briefly spin each Biotinylated Anti-Human IgA Antibody vial before use. Add 200 µl of 1X Assay Diluent B into each vial to prepare an antibody concentrate.
Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should then be diluted 100-fold with 1X Assay Diluent B and used in step 5 of Part VI Assay Procedure.

8. Briefly spin the HRP-Streptavidin concentrate (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 800-fold with 1X Assay Diluent B and used in step 7 of Part VI Assay Procedure.
For example: Briefly spin the vial and pipette up and down to mix gently. Add 25 µl of HRP-Streptavidin concentrate into a tube with 20 ml 1X Assay Diluent B to prepare an 800-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use. It is recommended that the positive control, and all samples be run at least in duplicate.

2. Label removable 8-well strips as appropriate for your experiment.

3. Add 100 µl of each prepared positive control (Positive Control, prepared in Reagent Preparation step 5), and sample (prepared in Reagent Preparation step 4) into appropriate wells of the SARS-CoV-2 S1 RBD protein coated 96 well-Microplate (Item A) and the Albumin protein coated 96 well-Microplate. Cover wells and incubate for 1 hour at room temperature with gentle shaking.

4. Discard the solution and wash 4 times with 1X Wash Buffer. Wash by filling each well with 300 µl of 1X Wash Buffer using a multi-channel Pipette or autowasher. Complete removal of all liquid at each step is essential for 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 prepared Biotinylated Anti-Human IgA Antibody (Item F, Reagent Preparation step 7) to each well. Incubate for 30 minutes at room temperature with gentle shaking.

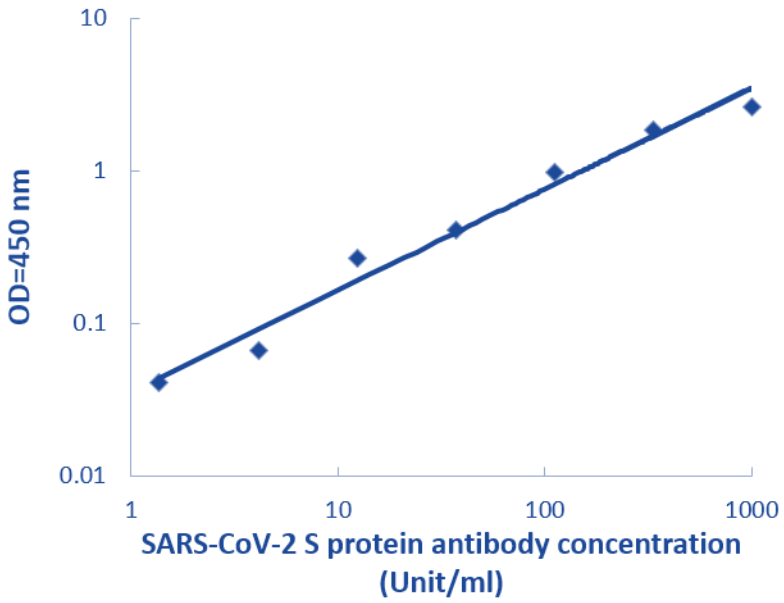
6. Discard the solution. Repeat the wash as in step 4.

7. Add 100 µl of prepared HRP-Streptavidin solution (see Reagent Preparation step 8) to each well. Incubate for 30 minutes at room temperature with gentle shaking.

8. Discard the solution. Repeat the wash as in step 4.

9. Add 100 µl of TMB One-Step Substrate Reagent to each well. Incubate for 15 minutes at room temperature in the dark with gentle shaking.

10. Add 50 µl of Stop Solution to each well. Read at 450 nm immediately.



Assay Procedure Summary

1. Prepare all reagents, samples and standards as instructed.

2. Add 100 µl positive control, or sample to each well. Incubate 1 hour at room temperature.

3. Add 100 µl prepared Biotinylated Anti-Human IgA Antibody into each well. Incubate 30 minutes at room temperature.

4. Add 100 µl prepared HRP-Streptavidin solution to each well. Incubate 30 minutes at room temperature.

5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 15 minutes at room temperature.

6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

Interpretation of Results

1. Subtract the signals of all wells of the Albumin protein coated plate from the signals of all wells of the S1 RBD coated plate, including positive control and samples, to remove the background.

2. Calibration curve: Calculate the mean absorbance for each set of duplicate Positive Control and samples from the background subtracted S1 RBD plate and then subtract the average zero Positive Control optical density. Plot the calibration curve on a log-log scale with Positive Control concentration (Unit/ml) on the x-axis and absorbance on the y-axis using SIgAa plot or Excel software. The following calibration curve is a typical data for demonstration only. A calibration curve must be run with each assay.

3. A positive result for an unknown sample is considered as a Unit/ml calculated value using the calibration curve of greater than 21.4 Unit/ml.

4. A negative result for an unknown sample is considered as a Unit/ml calculated value using the calibration curve of less than 21.4 Unit/ml

Troubleshooting

Problem	Causes	Solutions
Low signal	• Improper preparation of positive control and/or the HRP-conjugated antibodies. • Inadequate reagent volumes or improper dilution • Too brief incubation times	• Briefly spin down vials before opening. • Check pipettes and ensure correct preparation. • Ensure sufficient incubation time. Assay procedure step 3 may be done overnight at 4°C with gentle shaking (note: may increase overall signals including background).
Large CV	• Inaccurate pipetting • Air bubbles in wells	• Check pipettes • Remove bubbles in wells
High background	• Plate is insufficiently washed • Contaminated wash buffer	• Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed. • Make fresh wash buffer

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

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