RayBio® COVID-19 N and S1 RBD protein Human IgA ELISA Kit for Dried Blood Samples

Catalog #: IEQ-CoVSN-IgA-DBS

User Manual
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Caution:
Extraordinarily useful information enclosed

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RayBio® COVID19 N and S1 RBD protein Human IgA ELISA Kit Protocol for Dried Blood Samples

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Please read the entire manual carefully before starting your experiment

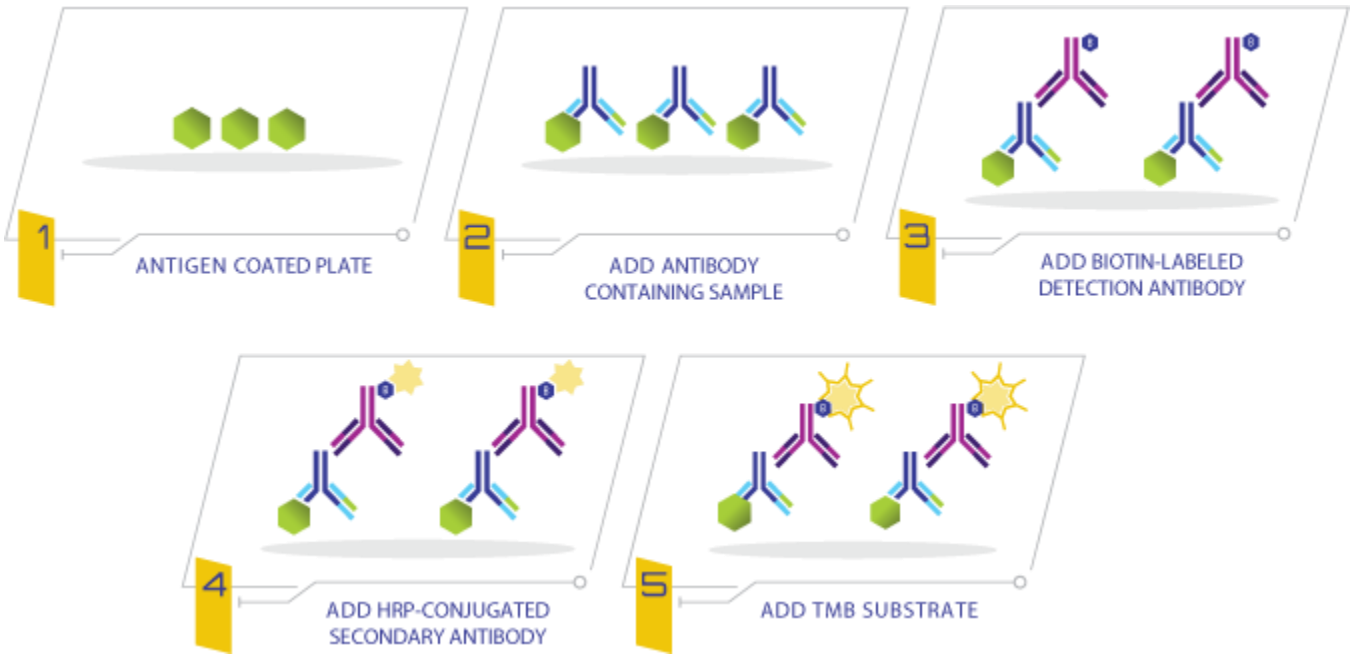
I. INTRODUCTION

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

PRINCIPLE OF THE ASSAY

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 N and S1 RBD proteins, 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 N and 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 a dried blood sample which contains SARS-COV-2 N and S1 RBD protein human IgA antibody. We do not know the exact amount of SARS-COV-2 N and 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.



II. STORAGE

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.

III. REAGENTS

Component	**Size / Description**	**Storage / Stability <u>After Preparation</u>**
SARS-CoV-2 N and S1 RBD protein coated 96 well-Microplate (Item A)	96 wells (12 strips x 8 wells) coated with SARS-CoV-2 N and S1 RBD protein	1 month at 4°C*
Albumin protein coated 96 well-Microplate (Item D)	96 wells (12 strips x 8 wells) coated with Albumin protein	1 month at 4°C*
Wash Buffer Concentrate (20X) (Item B)	40 ml of 20X concentrated solution.	1 month at 4°C
Positive Control (Item C)	2 vials of Positive Control sample from a dried blood sample which contains SARS-Cov-2 N and S1 RBD protein human IgA antibody.	1 week at -80°C
Biotinylated Anti-Human IgA (Item F)	2 vials of solution.	5 days at 4°C
HRP-Streptavidin concentrate (Item G)	1 vial of solution.	Do not store and reuse
TMB One-Step Substrate Reagent (Item H)	24 ml of 3,3,5,5'-tetramethylbenzidine (TMB buffer solution).	1 month at 4°C
Stop Solution (Item I)	16 ml of 0.2 M sulfuric acid.	N/A
Assay Diluent B (Item E)	15 ml of 5X concentrated buffer.	1 month at 4°C
5X Sample Diluent (Item J)	25 ml of 5X diluent buffer, 0.5% proclin 300 as preservative.	1 month at 4°C
1X PBS	15 ml of 1X PBS	1 month at 4°C
Tween-20	50 µl of Tween-20	N/A
Protease Inhibitor Cocktail	2 vials of lyophilized powder	1 month at -20 °C

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

IV. 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.

V. REAGENT PREPARATION

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

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

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

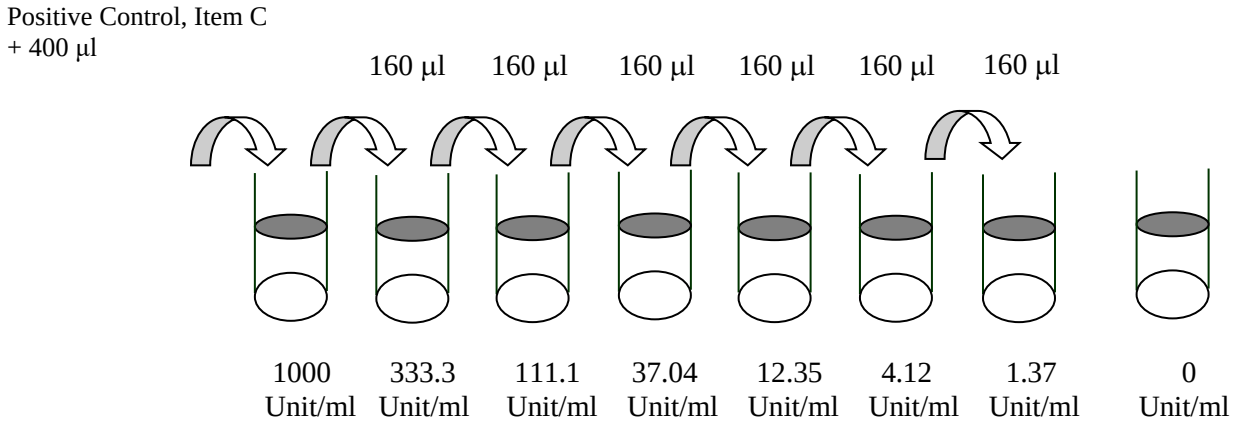
4. Preparation of the Elution Buffer:
    a. Add 60 µl of 1X PBS to each vial of Protease Inhibitor Cocktail to make a 100X stock solution, then resuspend each in 5.94 ml of 1X PBS for a total of 12 ml.
    b. Add 12 µl of Tween-20 to the 12 ml PBS plus Protease Inhibitor prepared in step 4a to make the Elution Buffer.

5. Elute the dried blood sample
    a. Elute the dried blood sample at a 1:10 ratio of elution buffer. (e.g. add 1 5x10mm strip from the PanoHealth® Blood Collection Device: PANO-BC-CD, or an equivalent 5x10mm area of a collected dried blood sample to 200 µl of Elution Buffer)
    b. Elute for 4h at room temperature on a shaker, vortexing for 10 seconds every 30 minutes
    c. Transfer the liquid to a clean tube leaving the filter paper behind.
    d. Centrifuge at 14000rpm for 10 minutes
    e. Collect the supernatant into a clean tube
    f. Dilute the collected supernatant 100-fold with 1X Sample Diluent. For example, add 5 µl of collected supernatant + 495 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.

6. Preparation of Positive Control calibration curve: **Briefly spin the vials of Positive Control, Item C.** Add 400 µl 1X Sample Diluent (Item J) into each Item C 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).



7. If the Wash Concentrate (20X) (Item B) 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.

8. Briefly spin the Biotinylated Anti-Human IgA Antibody vial (Item F) before use. Add 200 µl of 1X Assay Diluent B (Item E) 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

9. 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 (Item E) and used in step 7 of Part VI Assay Procedure.

   For example: Briefly spin the vial (Item G) 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 a 800-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

VI. 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 (Item C, prepared in Reagent Preparation step 5), and sample (prepared in Reagent Preparation step 4)

into appropriate wells of the SARS-CoV-2 N and S1 RBD protein coated 96 well-Microplate (Item A) and the Albumin protein coated 96 well-Microplate (Item D). 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.

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

11. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

VII. 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.

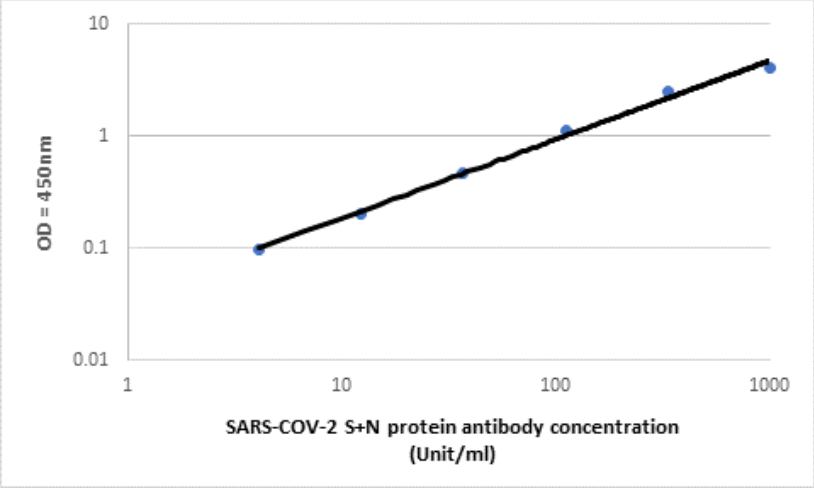
4. Add 100 µl prepared HRP-Streptavidin solution to each well. Incubate 30 minutes at room temperature.

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.

VIII. INTERPRETATION OF RESULTS

1. Subtract the signals of all wells of the Albumin protein coated plate from the signals of all wells of the N and 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 (Item C), and samples from the background subtracted N and 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 Sigma 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 2.56 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 2.56 Unit/ml.



IX. ASSAY PERFORMACE

1. The sensitivity of this assay against a reference standard is 34.21% (13/38, 95%CI: 19.63-51.35%).
2. The specificity of this assay against a reference standard is 86.56% (58/67, 95%CI: 76.02-93.66%).
3. The accuracy of this assay against a reference standard is 67.61% (71/105, 95%CI: 57.78-76.42%), with Kappa value of 0.4419 and the AUC=0.7663.

X. TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Low signal	Improper preparation of positive control and/or the HRP-conjugated antibodies	Briefly spin down vials before opening.
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Too brief incubation times	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	Check pipettes
	Air bubbles in wells	Remove bubbles in wells
High background	Plate is insufficiently washed	Review the manual for proper wash procedure. If using a plate washer, ensure that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer

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