RayBio® COVID-19 N protein Human IgA ELISA Kit

Catalog #: IE-CoVN-IgA

User Manual
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Caution:
Extraordinarily useful information enclosed

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RayBiotech, Inc.

RayBio® COVID19 Human IgA ELISA Kit Protocol

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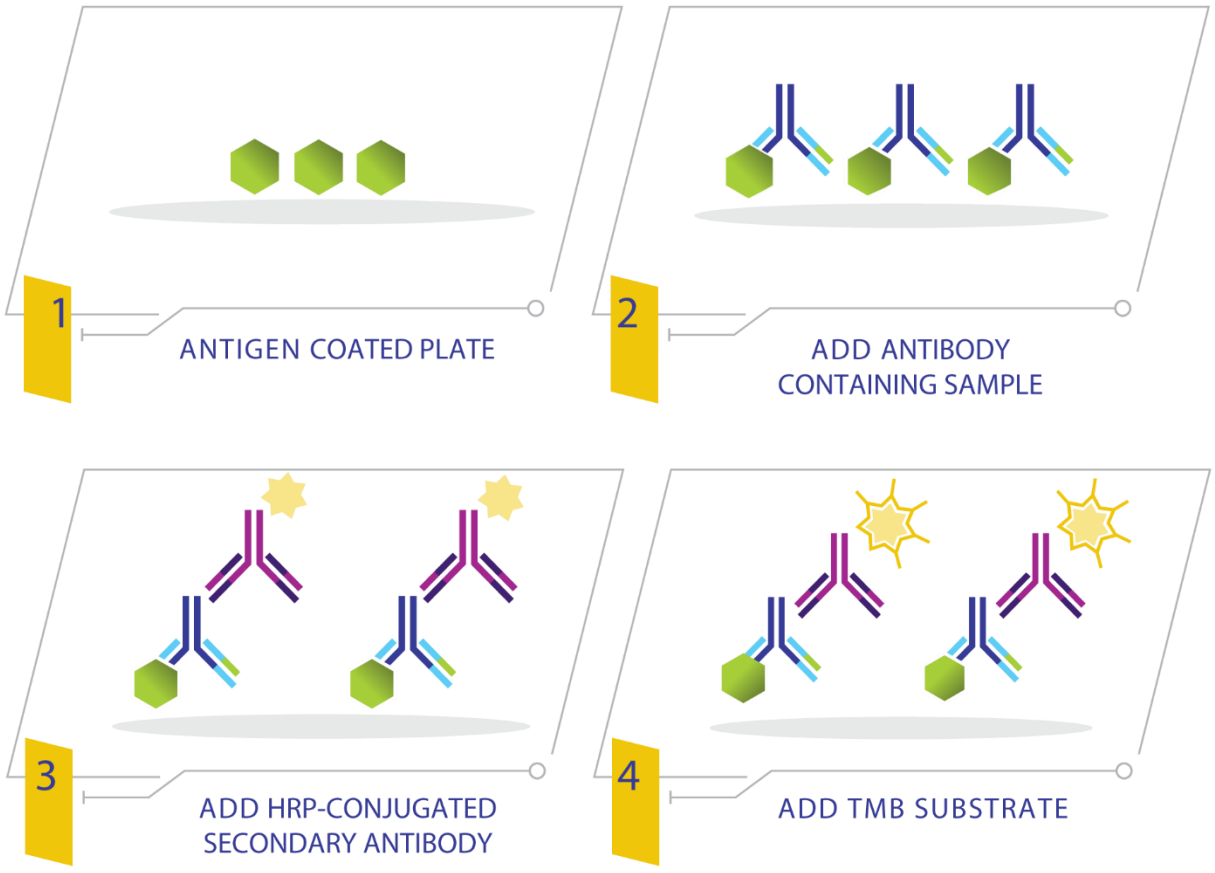
Please read the entire manual carefully before starting your experiment

I. INTRODUCTION

The Novel Coronavirus (SARS-CoV-2) N protein Human IgA ELISA Kit is an in vitro indirect ELISA for the semi-quantitative measurement of human IgA antibody against SARS-CoV-2 N protein in human serum or plasma. 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 protein, which combines with the corresponding antibody present in a sample. When a secondary anti-human Antibody-HRP is added, a complex of Antibody-HRP-human IgA antibody-virus N antigen forms on the microplate. A TMB substrate is added and a blue color is generated. The depth of color is relative to the amount of the anti-SARS-CoV-2 IgA antibody present. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.



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. The kit may be stored at 4°C for up to 6 months. 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 protein coated 96 well-Microplate (Item A)	96 wells (12 strips x 8 wells) coated with SARS-CoV-2 N protein	1 month at 4°C*
Wash Buffer Concentrate (20X) (Item B)	25 ml of 20X concentrated solution.	1 month at 4°C
Positive Control (Item C)	1 vial of Positive Control sample from an inactivated serum sample which contains SARS-CoV-2 N protein IgA antibody. 1 vial is enough to run 4 wells.	1 week at -80°C
Negative Control (Item C-2)	1 vial of solution without anti SARS-CoV-2 N protein antibody. 1 vial is enough to run 4 wells.	1 week at 4°C
HRP-anti IgA Antibody (Item F)	1 vial of solution.	5 days at 4°C
TMB One-Step Substrate Reagent (Item H)	12 ml of 3,3,5,5'-tetramethylbenzidine (TMB buffer solution)	1 month at 4°C
Stop Solution (Item I)	8 ml of 0.2 M sulfuric acid.	N/A
Assay Diluent B (5X) (Item E)	15 ml of a 5X concentrated buffer.	1 month at 4°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 Assay Diluent B (Item E) should first be diluted 5-fold with deionized water before use to make 1X Assay Diluent B (Item E).

3. Sample dilution: Dilute sample (human serum or plasma) with 1X Assay Diluent B (Item E) 500 times. For example, add 0.5µl serum + 249.5µl Assay 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, or contamination by bacteria or protein suspension.

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

4. Add 400 µl of 1X Assay Diluent B (Item E) into the Item C vial to prepare the Positive Control.

5. If the Wash Buffer Concentrate (20X) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.

6. Briefly spin the HRP-anti IgA Antibody vial (Item F) before use. Add 150 µl of 1X Assay Diluent B (Item E) into the vial to prepare a Detection Antibody Concentrate. Pipette up and down to mix gently. The Detection Antibody Concentrate should then be diluted 100-fold with 1X Assay Diluent B (Item E) and used in step 5 of Section VI Assay Procedure.

   Note: The Detection Antibody Concentrate can be stored at 4ºC for up to 5 days.

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 positive control (Item C), negative control (Item C-2) and sample (prepared in Reagent Preparation steps 3 and 4) into appropriate wells. Cover wells and incubate for 1.5 hours 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 HRP-anti IgA Antibody (Item F, Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

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

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

8. 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, negative control, or sample to each well. Incubate 1.5 hours at room temperature.

3. Add 100 µl prepared HRP- anti IgA Antibody to each well. Incubate 1 hour at room temperature.

4. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 10 minutes at room temperature.

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

VIII. INTERPRETATION OF RESULTS

1) Although a negative control is provided in the kit, it is recommended for the researcher to include at least 1 normal human serum/plasma sample as an additional negative control.

2) For the assay to be valid, the following specifications must be met: 1) the Positive Control mean optical density (PC: OD450) must be greater than 0.5. 2) the negative control(s) mean should be less than 0.3.

3) A positive result in an unknown sample is defined as an average OD which is 2 standard deviations above the negative control. If the researcher has provided their own negative control, they should use it in this calculation instead of the Negative Control (Item C-2).

4) The SARS-CoV-2 N protein coated in this kit is a recombinant protein expressed by mammalian cells. The antibodies used in this assay are polyclonal. The positive control in this kit is an inactivated serum sample containing SARS-CoV-2 N protein IgA antibody. This kit is not suitable for samples containing sodium azide (NaN3) which will affect the reactivity of HRP and result in the underestimation of the SARS-CoV-2 IgA Antibody levels.

VIIII. TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Low signal	Improper preparation of positive control and/or HRP-conjugated antibody	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.

This product is for research use only.