

Instructions for Use

Novel Coronavirus COVID-19 IgG ELISA RUO

RUO

REF EIA-6146R



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DRG 

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Please use only the valid version of the Instructions for Use provided with the kit.

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For Research Use Only

Not for use in diagnostic procedures

1 INTENDED USE

This kit is intended for the **qualitative detection** of human anti-COVID-19 IgG antibody in human serum.

2 INDICATIONS FOR USE

This kit is used as an aid for the detection of novel COVID-19.

This kit is for research use only; not for use in diagnostic procedures.

3 ASSAY PRINCIPLE

This ELISA kit is designed, developed, and produced for the qualitative measurement of the human anti-COVID-19 IgG antibody in serum. This assay utilizes the microplate based enzyme immunoassay technique.

Assay controls and 1:100 diluted human serum samples are added to the microtiter wells of a microplate that was coated with COVID-19 recombinant full length nucleocapsid protein.

After the first incubation period, the unbound protein matrix is removed with a subsequent washing step. A horseradish peroxidase (HRP) labeled polyclonal goat anti-human IgG tracer antibody is added to each well. After an incubation period, an immunocomplex of "COVID-19 recombinant antigen – human anti-COVID-19 IgG antibody - HRP labeled anti-human IgG tracer antibody" is formed if there is specific coronavirus IgG antibody present in the tested specimen.

The unbound tracer antibody is removed by the subsequent washing step. HRP-labeled tracer antibody bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the tracer antibody bound to the anti-COVID-19 IgG on the wall of the microtiter well is proportional to the amount of the anti-COVID-19 IgG antibody level in the tested specimen.

4 REAGENTS: PREPARATION AND STORAGE

This test kit must be stored at 2 °C to 8 °C upon receipt.

For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

1. COVID-19 antigen coated Microplate

Microplate coated with COVID-19 recombinant protein.

Qty: 1 x 96 well microplate

Storage: 2 °C - 8 °C

Preparation: Ready to use

2. COVID-19 IgG Sample Diluent

A ready-to-use sample dilution buffer.

Qty: 1 x 120 mL

Storage: 2 °C - 8 °C

Preparation: Ready to use.

3. HRP labeled Anti-IgG Tracer Antibody

HRP labeled polyclonal goat anti-human IgG antibody in a stabilized protein matrix.

Qty: 1 x 11 mL

Storage: 2 °C - 8 °C

Preparation: Ready to use.

4. ELISA Wash Concentrate

Surfactant in a phosphate buffered saline with non-azide preservative.

Qty: 1 x 30 mL

Storage: 2 °C - 25 °C

Preparation: 30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.

5. ELISA HRP Substrate

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.

Qty: 1 x 15 mL

Storage: 2 °C - 8 °C

Preparation: Ready to use.

6. ELISA Stop Solution

0.5 M sulfuric acid.

Qty: 1 x 15 mL

Storage: 2 °C - 25 °C

Preparation: Ready to use.

7. COVID-19 IgG Negative Control

Negative control with a bovine serum albumin based matrix with non-azide preservative.

Control products do not contain any serum from patients with new type of coronavirus infection.

Qty: 1 x 1 mL

Storage: 2 °C - 8 °C

Preparation: Ready to use.

8. COVID-19 IgG Positive Control

Positive control with a bovine serum albumin based matrix with non-azide preservative.

Control products do not contain any serum from patients with new type of coronavirus infection.

Qty: 1 x 0.5 mL

Storage: 2 °C - 8 °C

Preparation: Ready to use.

5 SAFETY PRECAUTIONS

The reagents are for research use only.

Source material which contains reagents of bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases.

Wear gloves while performing this assay and handle these reagents as if they were potentially infectious.

Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid.

Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes.

On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

6 MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 10 μ L, 25 μ L, 100 μ L, and 1000 μ L, etc.
2. Repeating dispenser suitable for delivering 100 μ L.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm or 13 x 100 glass tubes.
5. Disposable plastic 1000 mL bottle with caps.
6. Aluminum foil.
7. Deionized or distilled water.
8. Plastic microtiter well cover or polyethylene film.
9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

7 SAMPLE COLLECTION & STORAGE

Only 10 μ L of human serum is required for measurement in duplicate.

Samples should only be used on the same day.

Severe hemolytic samples should not be used.

8 ASSAY PROCEDURE

8.1 Reagent Preparation

1. Prior to use, allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate must be diluted to working solution prior to use. Please see REAGENTS section for details.

8.2 Sample Preparation

1. Dilute sample by a 1:100 dilution ratio with the COVID-19 IgG Sample Diluent.
For each 10 μ L of sample, 1000 μ L of COVID-19 IgG Sample Diluent is needed.
2. Mix well prior to performing the assay.

8.3 Assay Procedure

- Place a sufficient number of microwell strips in a holder to run controls and samples in duplicate.
- Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Negative Control	SAMPLE 3	SAMPLE 7
B	Negative Control	SAMPLE 3	SAMPLE 7
C	Negative Control	SAMPLE 4	SAMPLE 8
D	Positive Control	SAMPLE 4	SAMPLE 8
E	SAMPLE 1	SAMPLE 5	SAMPLE 9
F	SAMPLE 1	SAMPLE 5	SAMPLE 9
G	SAMPLE 2	SAMPLE 6	SAMPLE 10
H	SAMPLE 2	SAMPLE 6	SAMPLE 10

- Add **100 µL** of controls and **1:100 diluted samples** into the designated microwells.
- Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20 °C - 25 °C)** for **30 minutes**.
- Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
- Add **100 µL** of the HRP labeled Anti-IgG Tracer Antibody into the microwells.
- Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20 °C - 25 °C)** for **30 minutes**.
- Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
- Add **100 µL** of the substrate into the microwells.
- Mix gently and cover the plate with aluminum foil. Incubate at **room temperature (20 °C - 25 °C)** for **20 minutes**.
- Remove the aluminum foil and add **100 µL** of stop solution into each of the microwells. Mix by gently tapping the plate.
- Read the absorbance at **450 nm** within **10 minutes** with a microplate reader.

9 PROCEDURAL NOTES

- It is recommended that all samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
- Keep light-sensitive reagents in the original bottles and avoid unnecessary exposure to the light.
- Store any unused antibody-coated strips in the foil Ziploc bag with desiccant to protect from moisture.
- Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
- Incubation times or temperatures other than those stated in this insert may affect the results.
- Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
- All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

10 QUALITY CONTROL

To assure the validity of the results each assay must include both negative and positive controls.

The average value of the absorbance of the negative control is less than 0.25, and the absorbance of the positive control is **not less than 0.50**.

We also recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

11 INTERPRETION OF RESULTS







1. Calculate the average value of the absorbance of the negative control (xNC).
2. Calculate the Cutoffs using the following formulas:
 - Positive Cutoff = $1.1 \times (xNC + 0.18)$
 - Negative Cutoff = $0.9 \times (xNC + 0.18)$
3. Determine the interpretation of the sample by comparing the OD to the following table:

Interpretation	Interval	Results
Negative	Measured value \leq Negative Cutoff	The sample does not contain the new coronavirus (COVID-19) IgG related antibody.
Positive	Measured value \geq Positive Cutoff	The sample contains novel coronavirus (COVID-19) IgG associated antibodies.
Borderline	Negative Cutoff $<$ Measured value $<$ Positive Cutoff	Retest the sample.

12 LIMITATIONS OF THE PROCEDURE

1. This test is only for qualitative detection. For Research Use Only.
2. Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
3. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.
4. Not for the screening of donated blood.

SYMBOLS USED

Symbol	English
	Consult instructions for use
REF	Catalogue number
LOT	Batch code
	Contains sufficient for <n> tests
	Temperature limit
	Use-by date
	Manufacturer
	Caution
RUO	For research use only
<i>Distributed by</i>	Distributed by
<i>Content</i>	Content
<i>Volume/No.</i>	Volume / No.

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