



EDI™ Novel Coronavirus COVID-19 IgG ELISA Kit

Enzyme Linked Immunosorbent Assay (ELISA) for the qualitative detection of the COVID-19 IgG in human serum.

REF KT-1032 IVD CE

INTENDED USE

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

INDICATIONS FOR USE

This kit is used as an aid for the detection of novel COVID-19. Patients with suspected clustering cases require diagnosis or differential diagnosis of novel coronavirus infection. This kit is for in-vitro diagnostic use only.

SUMMARY OF PHYSIOLOGY

2019 novel coronavirus (COVID-19) is a single-stranded RNA coronavirus². Comparisons of the genetic sequences of this virus have shown similarities to SARS-CoV and bat coronaviruses⁷. In humans, coronaviruses cause respiratory infections³. Coronaviruses are composed of several proteins including the spike (S), envelope (E), membrane (M), and nucleocapsid (N)⁴. Results suggest that the spike protein retains sufficient affinity to the Angiotensin converting enzyme 2 (ACE2) receptor to use it as a mechanism of cell entry⁶. Human to human transmission of coronaviruses is primarily thought to occur among close contacts via respiratory droplets generated by sneezing and coughing¹. IgG is the most abundantly found immunoglobulin to be produced in response to an antigen and will be maintained in the body after initial exposure for long term response⁵.

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.

REAGENTS: PREPARATION AND STORAGE

This test kit must be stored at 2 – 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 (31217)

Microplate coated with COVID-19 recombinant protein.

Qty: 1 x 96 well microplate

Storage: 2 – 8°C

Preparation: Ready to use.

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2. COVID-19 IgG Sample Diluent (31218)

A ready-to-use sample dilution buffer.

Qty: 1 x 120 mL

Storage: 2 – 8°C

Preparation: Ready to use.

3. HRP labeled Anti-IgG Tracer Antibody (31220)

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

Qty: 1 x 11 mL

Storage: 2 – 8°C

Preparation: Ready to use.

4. ELISA Wash Concentrate (10010)

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

Qty: 1 x 30 mL

Storage: 2 – 25°C

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

5. ELISA HRP Substrate (10020)

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.

Qty: 1 x 15 mL

Storage: 2 – 8°C

Preparation: Ready to use.

6. ELISA Stop Solution (10030)

0.5 M sulfuric acid.

Qty: 1 x 15 mL

Storage: 2 – 25°C

Preparation: Ready to use.

7. COVID-19 IgG Negative Control (31221)

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 – 8°C.

Preparation: Ready to use.

8. COVID-19 IgG Positive Control (31222)

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 – 8°C.

Preparation: Ready to use.

SAFETY PRECAUTIONS

The reagents are for in-vitro diagnostic 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.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 10 µL, 25 µL, 100 µL, and 1000 µL, etc.
2. Repeating dispenser suitable for delivering 100 µL.
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.

SAMPLE COLLECTION & STORAGE

Only 10 µL 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.

ASSAY PROCEDURE

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 (10010) must be diluted to working solution prior to use. Please see REAGENTS section for details.

2. Sample Preparation

1. Dilute sample by a 1:100 dilution ratio with the COVID-19 IgG Sample Diluent (31218). For each 10 µL of sample, 1000 µL of COVID-19 IgG Sample Diluent (31218) is needed.
2. Mix well prior to performing the assay.

3. Assay Procedure

1. Place a sufficient number of microwell strips (31217) in a holder to run controls (31221, 31222) and samples in duplicate.
2. 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 |

3. Add **100 µL** of controls (31221, 31222) and 1:100 diluted samples into the designated microwells.
4. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 °C)** for **30 minutes**.
5. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
6. Add **100 µL** of the HRP labeled Anti-hlgG Tracer Antibody (31220) into the microwells.
7. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 °C)** for **30 minutes**.
8. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
9. Add **100 µL** of the substrate (10020) into the microwells.
10. Mix gently and cover the plate with aluminum foil. Incubate at **room temperature (20-25 °C)** for **20 minutes**.
11. Remove the aluminum foil and add **100 µL** of stop solution (10030) into each of the microwells. Mix by gently by tapping the plate.
12. Read the absorbance at **450 nm** within **10 minutes** with a microplate reader.

PROCEDURAL NOTES

1. 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.
2. Keep light-sensitive reagents in the original bottles and avoid unnecessary exposure to the light.
3. Store any unused antibody-coated strips in the foil Ziploc bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
7. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

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.

INTERPRETION OF RESULTS

1. Calculate the average value of the absorbance of the negative control (xNC).
2. Calculate the Background Adjustment Factor (BAF) using the following formulas:
 - $BAF = xNC - 0.10$
3. Subtract the Background Adjustment Factor from all of the ODs of the unknown samples.
4. The fixed positive cut off is 0.22 and negative cut off is 0.18. Determine the interpretation of the sample by comparing the OD to the following table:

| Interpretation | Interval | Results |
|----------------|------------------------------|---|
| Negative | Measured value \leq 0.18 | The sample does not contain the new coronavirus (COVID-19) IgG related antibody |
| Positive | Measured value \geq 0.22 | The sample contains novel coronavirus (COVID-19) IgG associated antibodies. |
| Borderline | 0.18 < Measured value < 0.22 | Retest the sample in conjunction with other clinical tests. |

LIMITATIONS OF THE PROCEDURE

1. This test is only for qualitative detection. Test results should not be the sole basis for clinical diagnosis and treatment. The confirmation of infection with novel coronavirus (COVID-19) must be combined with the patient's clinical signs in conjunction to other tests.
2. In the first week of the onset of the infection with the novel coronavirus (COVID-19) patients results may be negative for IgG. In addition, patients with low immunity or other diseases that affect immune function, failure of important systemic organs, and use of drugs that suppress immune function can also lead to negative results of new coronavirus IgG. Previous infection of SARS or other coronavirus strain may cause a light IgG positive in view of similarity of different strains.
3. Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
4. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

PERFORMANCE CHARACTERISTICS

Limit of Detection

The limit of detection is not higher than 5 U/mL

Repeatability

The assay control is tested in 10 replicates with a CV of OD values less than 15%.

Reproducibility

Three lots were tested with the same samples 10 times with a CV less than 20%.

CLINICAL TESTING

Serum samples from two cohorts of patients were tested using the IgG ELISA kit at the Jiaxing City Center for Disease Control and Prevention and Zhejiang University Hospital. The combined cohort consisted of normal healthy patients with samples collected prior to the COVID-19 outbreak [December 3, 2019] (n = 54) and RT-PCR confirmed positive patients in after the second week of the onset of the disease (n = 30). The results are as follows:

| | Test Positive | Test Negative |
|--------------------|---------------|---------------|
| Confirmed Positive | 30 | 0 |
| Confirmed Negative | 0 | 54 |

The diagnostic sensitivity is 100%.

The diagnostic specificity is 100%.

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE













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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)

| | | |
|---|--|---|
|  In Vitro Diagnostic Device |  For Research Use Only |  Lot Number |
|  Catalog Number |  Read instructions before use |  Number of Tests |
|  Store at |  Use by |  Keep away from heat and direct sun light |
|  Manufacturer |  Authorized Representative in Europe |  European Conformity |

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