EPITOPE DIAGNOSTICS, INC.

EDI™ Novel Coronavirus 2019-nCoV IgG ELISA Kit

Enzyme Linked Immunosorbent Assay (ELISA) for the qualitative detection of the 2019-nCoV IgG in serum or plasma.

REF KTR-1032 RUO

INTENDED USE

This kit is used for the qualitative detection of novel coronavirus-infected pneumonia cases, patients with suspected clustering cases, and other new coronaviruses in serum or plasma samples (2019-nCoV) that require diagnosis or differential diagnosis of new coronavirus infections through measurement of the 2019-nCoV IgG antibody. This kit is for research use only.

SUMMARY OF PHYSIOLOGY

ASSAY PRINCIPLE

This ELISA kit is designed, developed, and produced for the qualitative measurement of the 2019-nCoV IgG antibody in serum or plasma samples. This assay utilizes the microplate based enzyme immunoassay technique.

Assay controls and samples are added to the microtiter wells of a microplate that was coated with the 2019-nCoV peptide antigen along with a coronavirus IgG antibody. After the first incubation period, the unbound protein matrix is removed with a subsequent washing step. A horseradish peroxidase labeled 2019-nCoV IgG tracer antibody is added to each well. After an incubation period, an immunocomplex of " 2019-nCoV polypeptide antigen - new coronavirus IgG antibody - HRP labeled 2019-nCoV IgG tracer antibody " is formed if there is coronavirus IgG antibody present in the tested materials. 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 coronavirus IgG on the wall of the microtiter well is proportional to the amount of the coronavirus IgG antibody level in the tested materials.

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. 2019-nCoV antigen coated Microplate (31217)

Microplate coated with 2019-nCoV peptide antigen.

Qty: 1 x 96 well microplate
Storage: 2 – 8°C
Preparation: Ready to use

2. 2019-nCoV IgG Sample Diluent (31218)

Upon dilution, this yields a ready-to-use sample dilution buffer.

Qty: 1 x 30 mL
Storage: 2 – 8°C
Preparation: 5X concentrate. The contents must be diluted with 120 mL distilled water and mixed well before use.

3. IgG Tracer Antibody Diluent (31219)

Buffer for antibody dilution according to assay procedures.

Qty: 1 x 11 mL
Storage: 2 – 8°C
Preparation: Ready to use.

4. HRP labeled 2019-nCoV IgG Tracer Antibody (31220)

HRP labeled 2019-nCoV IgG antibody in a stabilized protein matrix.

Qty: 1 x 250 µL
Storage: 2 – 8°C
Preparation: 50X concentrate. The contents must be diluted with IgG Tracer Antibody Diluent (31219) prior to use.

5. 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.

6. ELISA HRP Substrate (10020)

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.

Qty: 1 x 15 mL
Storage: 2 – 8°C
Preparation: Ready to use.

7. ELISA Stop Solution (10030)

0.5 M sulfuric acid.

Qty: 1 x 15 mL
Storage: 2 – 25°C
Preparation: Ready to use.

8. 2019-nCoV 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.

9. 2019-nCoV 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 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.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 20 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 or plasma 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.
3. 2019-nCoV IgG Sample Diluent (31218) must be diluted to working solution prior to use. Please see REAGENTS section for details.
4. HRP labeled 2019-nCoV IgG Tracer Antibody (31220) must be diluted to the tracer antibody 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 diluted 2019-nCoV IgG Sample Diluent (31218). For each 10 µL of sample, 1000 µL of diluted 2019-nCoV 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	SAMPLE4	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 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 ºC)** 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 tracer antibody working solution into the microwells.
7. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** 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 one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **20 minutes.**
11. Remove the aluminum foil and plate sealer and add 100 µL of stop solution (10030) into each of the microwells. Mix by gently 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.

INTERPRETION OF RESULTS

Interpretation	Interval	Results
Negative	Measured value ≤ negative limit value	The sample does not contain the new coronavirus (2019-nCoV) IgG- related antibody
Positive	Measured value ≥ positive limit value	The sample contains novel coronavirus (2019-nCoV) an IgG -associated antibodies.
Borderline	Negative limit value < Measured value < Positive limit value	Retest the sample in conjunction with other clinical tests.

LIMITATIONS OF THE PROCEDURE

1. This test is only for qualitative detection and diagnosis and should not be the sole basis for clinical diagnosis and treatment. The infection is confirmed novel coronavirus (2019-nCoV) must be combined with the patient's clinical signs in conjunction to other tests.
2. Infection novel coronavirus (2019-nCoV) patients the first week of the onset of which novel coronavirus IgG may be negative. In addition, patients with low autoimmunity or other diseases that affect autoimmune function, failure of important systemic organs, and use of drugs that suppress immune function can also lead to negative results of new coronavirus IgG.
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 inactive the horseradish peroxidase enzyme.

QUALITY CONTROL

To assure the validity of the results each assay must include both negative and positive controls. We also recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

PERFORMANCE CHARACTERISTICS

Limit of Detection

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

Repeatability

The assay was repeated 10 times with a CV less than 15%.

Reproducibility

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

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.

REFERENCES

1. Chenjia Yuan , Shi Jinsong , Qiudong An , Liu Chang , Li Xin , Qiang , Ruanji Shou , mountains . Wuhan 2019 Bioinformatics coronavirus genome analysis [J / OL]. Bioinformatics : 1-10 [2020-02-10].

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.

This product is developed and manufactured by

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)