EPITOPE DIAGNOSTICS, INC.

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 COVID-19 IgG antibody in human serum. It detects IgG antibody specific binding to SARS-CoV-2 Nucleocapsid protein and doesn't detect antibody to Spike protein in human serum. It is for screening or to aid in the diagnosis of COVID-19. Patients with suspected clustering cases require diagnosis or differential diagnosis of novel coronavirus infection. The assay is validated manually, but can be adapted to an automated instrument.

For In-vitro diagnostic Purpose Only

INTENDED USER

This kit is for laboratory or healthcare professionals.

SUMMARY OF PHYSIOLOGY

2019 novel coronavirus (COVID-19) is a single-stranded RNA coronavirus[2]. Comparisons of the genetic sequences of this virus have shown similarities to SARS-CoV and bat coronaviruses[7]. In humans, coronaviruses cause respiratory infections[3]. 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 (ACE-2) receptor to use it as a mechanism of cell entry[6]. Human to human transmission of coronaviruses is primarily thought to occur among close contacts via respiratory droplets generated by sneezing and coughing[1]. 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 response[5].

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

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-hIgG Tracer Antibody (31273)
Concentrated (21x) HRP labeled polyclonal goat anti-human IgG antibody in a stabilized protein matrix.
Qty: 1 x 0.6 mL
Storage: 2 – 8°C
Preparation: 1:21 dilution before use

4. Tracer Antibody Diluent (31274)
A buffer matrix for diluting the HRP labeled Anti-hIgG Tracer Antibody.
Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Mix with the 21x HRP labeled anti-hIgG tracer antibody before assay.

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 Concentrated. Must be diluted with 870 mL of distilled water and mix well before use.

6. ELISA HRP Substrate (10020)
Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.
Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to use

7. ELISA Stop Solution (10030)
0.5 M Sulfuric Acid.
Qty: 1 x 12 mL
Storage: 2 – 25°C
Preparation: Ready to use

8. COVID-19 IgG Negative Control (31221)
Negative control with a bovine serum albumin based matrix with non-azide preservative.
Qty: 1 x 1 mL
Storage: 2 – 8°C
Preparation: Ready to use

9. COVID-19 IgG Positive Control (31222)
Positive control with a bovine serum albumin based matrix with non-azide preservative.
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. Keep out of reach skin, eyes and/or clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Exercise 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 mm plastic 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 multichannels wash bottle or automatic (semi-automatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.
11. Calibrated Timer.

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.
3. Dilute the HRP Labeled anti-hIgG Tracer Antibody (31273) 1:21 with Tracer Antibody Diluent (31274) by pipetting **0.6 mL** Tracer antibody to the diluent bottle.

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 the negative control (31221) in triplicate, the positive control (31222) in singlet, 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 (20-25ºC)** 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 1:21 diluted HRP labeled Anti-hIgG Tracer Antibody into the microwells.
7. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25ºC)** 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 (20-25ºC)** for **20 minutes.**
11. Remove the aluminum foil and add **100 µL** of stop solution (10030) into each of the microwells. Mix 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 away from direct light in the original container and should be stored in a dark area avoiding unnecessary exposure to the light.
3. Store any unused antibody-coated strips in the foil ziploc bag with desiccant to protect from the moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation time(s) and/or temperature(s) other than those specified in the package insert may affect result(s).
6. Avoid air bubbles in the microwell as it could result in lower binding efficiency and higher CV% of a duplicate reading.
7. All reagents should be mixed thoroughly and gently 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 0.30. EDI recommends to include own laboratory controls in addition to those provided with the kit.

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 x (xNC + 0.18)
   - Negative cutoff = 0.9 x (xNC+ 0.18)
3. Determine the interpretation of the sample by comparing the OD to the following table:

Interpretation	Interval	Results
Negative	Measured value ≤ Negative Cut-off	No COVID-19 IgG antibody was found
Positive	Measured value ≥ Positive Cut-off	COVID-19 IgG antibody was found
Borderline	Negative Cut-off < Measured value < Positive Cut-off	Retest the sample in conjunction with other clinical tests

Race and geographical region may affect the results from normal donor samples. Laboratories may establish or modify the cutoff based upon additional validation.

LIMITATIONS OF THE PROCEDURE

1. This kit is for qualitative detection and in-vitro diagnostic use only, and should not be served as a sole basis for clinical diagnosis and patient treatment plan. The confirmation of infection with novel coronavirus (COVID-19) must be combined with the patient's clinical signs in conjunction to other clinical tests.
2. In the first week of infection and symptoms onset with novel coronavirus (COVID-19), results may be negative for IgG. In addition, low immunity or diseases that affect immune function, important systemic organ failure, or use of drugs that suppress immune functions are conditions that may contribute to negative results of new coronavirus IgG. Previous infection of SARS or other coronavirus strains may show light IgG positive results.
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

Assay Development

This assay was developed by evaluating eight commercially available COVID-19 antigens to screen for optimal use in this serological test. The assays were first evaluated with normal healthy donor serum samples to obtain negative test results. The assays were further evaluated with 20 positive serum samples from confirmed COVID-19 patients tested by RT-PCR. The best performing antigen was selected for the development of the kit. During this time, cross-reactivity was identified, but eliminated during the final selection of the antigen.

Reactivity/Inclusivity

Although mutations in the SARS-CoV-2 genome have been identified as the virus has spread, no serologically unique strains have been described relative to the originally isolated virus (this research is exceptionally limited at present).

Limit of Detection

Three lots of material were tested with one assay using a blank control in sixteen replicates. The LoD was calculated as the mean of the OD for the blank control plus three times the standard deviation. The highest value of the three runs was established for the LoD at 0.0666.The results are as follows:

Run	Average OD at 450 nm	CV (%)	LOD (x̄ + 3 SD)
1	0.0481	4.83%	0.0550
2	0.0518	5.71%	0.0606
3	0.0531	8.44%	0.0666

Repeatability

One lot of material was tested with one assay using three samples (strong positive, light positive, and negative) in sixteen replicates. For all sixteen replicates, sample 1 and 2 are positive and in sample 3 is all negative. The repeatability results are very satisfactory with acceptable CV. The results are as follows:

Sample	Average OD at 450 nm	CV (%)	Results
1	1.071	6.35%	16/16 (+)
2	0.631	3.11%	16/16 (+)
3	0.199	4.99%	16/16 (-)

Reproducibility

One lot of material was tested over twelve assays using three samples (strong positive, light positive, and negative) in a duplicate and a set of positive and negative controls in a triplicate. For all twelve assays, sample 1 and 2 are positive samples and sample 3 is negative sample. The results are as follows:

Sample	Average OD at 450 nm	CV (%)	Results
1	1.11	1.96%	12/12 (+)
2	0.65	3.47%	12/12 (+)
3	0.19	4.66%	12/12 (-)
Negative Control	0.17	3.15%	12/12 (-)
Positive Control	0.65	4.14%	12/12 (+)

Class Specificity

To evaluate class specificity, Ten (10) RT-PCR confirmed COVID-19 patients' serum samples were tested in a duplicate using EDI's qualitative IgG and IgM ELISA Kits. Sample 1 - 5 are IgM positive and IgG negative. Sample 1 is a natural and untreated IgM positive and IgG negative. Sample 2 - 5 were originally positive for IgG and IgM but used protein A/ProSep A to remove the IgG. Samples 6 -10 are IgG positive and IgM negative. Samples 6 - 10 are natural and untreated IgG positive and IgM negative. There is 100% agreement between the results of this test. This demonstrates that the assay is specific to the detection of IgG antibodies without cross reaction to COVID-19 IgM antibodies. The results are as follows:

Sample	IgM Result	IgG Result	Sample	IgM Result	IgG Result
1	+	-	6	-	+
2	+	-	7	-	+
3	+	-	8	-	+
4	+	-	9	-	+
5	+	-	10	-	+

Cross-Reactivity

A large number of known negative samples (N=570) collected in the US prior to December 2019 were tested from a population with a high prevalence of vaccination against, and/or infection with the following viruses, and specificity of 99.8% is observed, cross-reactivity for the following viruses is not expected at this time:

Anti-influenza A (IgG & IgM)	Anti-NL63 (α-coronavirus)
Anti-influenza B (IgG & IgM)	Anti-OC43 (β-coronavirus)
Anti-HBV (IgG & IgM)	Anti-HKU1 (β-coronavirus
Anti-HCV (IgG & IgM)	ANA (Antinuclear antibody)
Anti-haemophilus influenza (IgG & IgM)	Anti-respiratory syncytial virus (RSV) (IgG & IgM)
Anti-229E (α-coronavirus)	Anti-HIV

To demonstrate cross-reactivity of the test, five individual samples were tested in duplicate for each disease/infectious agent using natural specimen confirmed with commercially available diagnostic tests. All samples were sourced from natural specimens using sera from patients with the underlying diseases in the acute or convalescent stages of infection. Due to unavailability of the Anti-haemophilus influenzae and rhinovirus were not tested.

Agent	Disease State Confirmation Tests	Results
Influenza A	Viron/Serion	5/5 Negative
Influenza B	Viron/Serion	5/5 Negative
Respiratory Syncytial Virus	EIA, Viron/Serion	5/5 Negative
Hepatitis B Virus	Siemens	5/5 Negative
Hepatitis C Virus	Roche-Ampliprep/Taqman	5/5 Negative
Antinuclear Antibodies	Bio-Rad Hep 2	5/5 Negative

Transportation Stability

One lot of material was shipped from Epitope Diagnostics, Inc. in San Diego, CA to an external site in the United States and returned. The kit was packaged in a foam box with blue ice which was not changed for the duration of the study to simulate transport conditions. The kits were in this condition for a total of 12 days. A comparison of the values obtained before and after shipment demonstrates the stability of the materials. The results are as follows:

Before Shipment			After Shipment		
Well ID	**OD**	**Average**	**Well ID**	**OD**	**Average**
Negative	0.145	0.142	Negative	0.110	0.110
	0.146			0.113	
	0.134			0.107	
Positive	0.588	0.588	Positive	0.526	0.526
Negative Cut-off		0.290	Negative Cut-off		0.261
Positive Cut-off		0.354	Positive Cut-off		0.319

CLINICAL TESTING

Patient samples were tested using the EDI's qualitative IgG ELISA kit at four sites. The combined cohort consisted of normal healthy patients with samples collected prior to the COVID-19 outbreak (N=624) and RT-PCR confirmed positive patients (N=187). The results are as follows:

EDI™ Novel Coronavirus COVID-19 IgG ELISA Kit		Positive Samples	Normal Samples
KT-1032	Positive	184	1
	Negative	3	623
	Total	187	624
PPA: 98.4%	95% CL (Wilson's Score): 0.954 – 0.995		
NPA: 99.8%	95% CL (Wilson's Score): 0.991 – 0.997		

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. CDC (2020). Transmission of Novel Coronavirus (COVID-19).
2. 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].
3. Fehr, A. R., & Perlman, S. (2015). Coronaviruses: An Overview of Their Replication and Pathogenesis. *Coronaviruses Methods in Molecular Biology*, 1–23. doi: 10.1007/978-1-4939-2438-7_1
4. Li, F., Li, W., Farzan, M., & Harrison, S. (2005). Structure of SARS coronavirus spike receptor-binding domain complexed with its receptor. doi: 10.2210/pdb2ajf/pdb
5. Wu, L.-P., Wang, N.-C., Chang, Y.-H., Tian, X.-Y., Na, D.-Y., Zhang, L.-Y., Liang, G.-D. (2007). Duration of Antibody Responses after Severe Acute Respiratory Syndrome. Emerging Infectious Diseases, 13(10), 1562–1564. doi: 10.3201/eid1310.070576
6. Xu, X., Chen, P., Wang, J., Feng, J., Zhou, H., Li, X., Hao, P. (2020). Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Science China Life Sciences*. doi: 10.1007/s11427-020-1637-5
7. Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Shi, Z.-L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. doi: 10.1038/s41586-020-2012-7

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at +1 (858) 693-7877, fax to +1 (858) 693-7678 or email at cs@epitopediagnostics.com

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)