COVID-19 IgG ELISA

CAT NO	DESCRIPTION	PACK SIZE
EIACVG1	COVID-19 IgG ELISA	96 Tests

Intended Use:

The COVID-19 IgG ELISA is an enzyme linked immunosorbent assay (ELISA) for the qualitative detection of Immunoglobulin G to COVID-19 in human serum and plasma. It is intended for screening of patients suspected of infection with coronavirus disease 2019 (COVID-19). This reagent is for In vitro diagnostic use only.

Summary and Principle:

Coronaviruses are a large family of viruses that cause disease ranging from common cold symptoms to more severe pneumonia. They are enveloped, single strand RNA viruses. Coronaviruses are zoonotic, they can be transmitted from animals to humans. Existing examples include the Middle East Respiratory Virus (MER-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV).

Reports of a novel coronavirus began in the Wuhan district of China in December 2019 and in February 2020 the World Health Organisation designated the disease caused by the new strain as coronavirus disease 2019 (COVID-19). Symptoms include high temperature, cough and breathing difficulties. In immunocompromised individuals symptoms can be more severe leading to pneumonia, severe acute respiratory syndrome or death.

For detection of anti-SARS-CoV-2 IgG, the COVID-19 IgG ELISA uses a sandwich ELISA method in which wells are pre-coated with recombinant SARS-CoV-2 antigen. Samples are added to and incubated in assigned wells. Any anti-SARS-CoV-2 antibodies present in the samples will bind to the immobilised antigen. The plate is washed to remove unbound sample components. Next conjugate, consisting of anti-human IgG conjugated to Horse Radish Peroxidase (HRP), is added to the wells and incubated. Of the anti-SARS-CoV-2 antibodies bound during the first step, specifically anti-SARS-CoV-2 IgG will interact with and be labelled by the conjugate. Unbound conjugate is removed in another wash step. Then Chromogen solutions containing tetramethyl-benzidine (TMB) and urea peroxide are added to the wells where the HRP label on the immunocomplexes catalyses a reaction in the chromogens to form a blue coloured product. The blue colour turns yellow after stopping the reaction with acidic reagent. The colour intensity of the wells is measured spectrophotometrically and is proportional to the amount of antibody in the sample. Wells containing samples negative for anti-SARS-CoV-2 IgG remain colourless.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each well is coated with COVID-19 antigen. The 8 well strips can be broken and used individually. Place unused wells or strips in the plastic sealable bag together with the desiccant provided and store at 2 - 8°C. Once open the wells are stable for one month at 2 - 8°C.
Negative Control	1x1ml	Protein stabilized buffer which tests non-reactive for anti-SARS-CoV-2. Pale yellow in colour. Ready to use. Once open stable for one month at 2 - 8°C.
Positive Control	1x1ml	Protein stabilized buffer containing anti-SARS-CoV-2 IgG. Red coloured solution. Once open, stable for one month at 2 - 8°C.
Sample Diluent	1x12ml	Green coloured liquid. Serum based buffer containing casein and sucrose.
Conjugate	1x12ml	Red coloured liquid. HRP conjugated anti-human IgG. Once open, stable for one month at 2 - 8°C.
Wash Buffer (20X)	1x40ml	PBS at pH 7.4, 20X concentrate. The concentrate must be diluted 1/20 with distilled water before use. Once diluted it is stable at room temperature for a week or two weeks at 2 - 8°C.
Substrate Solution A	1x6ml	Urea peroxide solution. Ready to use. Once open, stable for one month at 2 - 8°C.
Substrate Solution B	1x6ml	TMB Solution. Ready to use. Once open, stable for one month at 2 - 8°C.
Stop Solution	1x6ml	0.5M Sulphuric acid solution. Ready to use. Once open, stable for 1 month at 2 - 8°C.

Plastic Sealable bag, plate covers and IFU.

Materials required but not provided:

Distilled water or deionized water, PPE, automatic pipettes, disposable pipette tips, absorbent tissue, dry bath incubator or water bath, plate reader (single wavelength 450nm or dual wavelength 450/630nm), timer and automatic plate washer.

Specimen Collection:

Collect serum and EDTA, sodium citrate or heparin plasma by separation from red blood cells after standard venepuncture technique. For serum, the blood must be allowed to clot fully but be separated as soon as possible to avoid haemolysis of the RBC. Any visible particulate matter in the serum or plasma should be removed by centrifugation. Do not heat inactivate samples.

Samples will be stable for analysis up to 7 days at 2 - 8°C or stored at -20°C.

Do not use samples with high content of haemoglobin, bilirubin or triglyceride as these substances can interfere in the ELISA.

The COVID-19 IgG ELISA assay is used only for testing serum or plasma samples. Do not use for testing cadaver samples, saliva, urine or other body fluids or pooled blood.

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored at 2-8°C. Do not freeze. Keep all components tightly capped and without any contamination.

Precautions and Safety:

The ELISA assays are time and temperature sensitive. To avoid incorrect results, follow the test procedure exactly as specified.

1. **Do not mix reagents from different batches or substitute reagents from other commercially available kits. The components of the kit are carefully matched for optimal performance of the assay.**
2. **Make sure that all reagents are within the expiry indicated on the kit box and of the same lot. Never use reagents beyond the expiry date stated on labels.**
3. **IMPORTANT: Allow all reagents and samples to reach room temperature (15 - 30°C) before use. Shake each reagent gently before use. Return at 2 - 8°C immediately after use.**
4. **Do not touch the bottom of the plate; fingerprints or scratches can interfere with the absorbance measurement. When reading the results, ensure that the plate bottom is dry and that all wells are devoid of air bubbles.**
5. **Never allow the wells to dry after a washing step. Immediately carry out the next step.**
6. **Make sure timing of reagent additions are the same for all wells and for each procedure step.**
7. **Use a new pipette tip for each sample and reagent in order to avoid cross-contamination. Touch the pipette tip against the side of the well to prevent formation of air bubbles and never touch the bottom of the well.**
8. **The activity of the HRP-conjugate is easily contaminated by sodium hypochlorite, acids and alkalis. Do not let these substances contaminate the Conjugate reagent.**
9. **If using a fully automated ELISA analyser do not cover the plate with a plate cover during incubation. Follow the manufacturers User Manual.**
10. **All samples from human origin should be considered as potentially infectious. Strict adherence to Good Laboratory Practice regulations will ensure personal safety.**
11. **WARNING: Materials from human origin may have been used in the preparation of the Negative Control of the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV 1/2, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the samples or reagents are completely absent. Therefore, handle reagents and samples with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.**
12. **Solid consumable waste (pipette tips, vials, well strips and sample containers) should be autoclaved for not less than 2 hours at 121oC and all liquid waste treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should never be autoclaved. MSDS available upon request. Reagents must be disposed of only in accordance with local or national regulations**
13. **Some reagent components may cause toxicity, irritation, burns or have carcinogenic effects as raw materials. Avoid contact of all reagents with skin and the mucosa but especially the Stop Solution, Substrate reagents and the Wash Buffer.**
14. **The Stop Solution contains sulphuric acid. Use it with appropriate care. Wipe up spills, immediately and wash with water if comes into contact with the skin or the eyes.**
15. **Proclin 300 is used as preservative and can cause a reaction on the skin. Wipe up spills immediately or wash with water if comes into contact with skin or eyes.**
16. **Indicators of deterioration of the reagents: The values of Positive and Negative controls fall out of the quality control range may indicate reagent instability or operator or equipment error. Where quality controls are out of range the results should be considered invalid and the samples must be retested. In case of consistently erroneous results and proven deterioration of the reagents, immediately discard the reagents in use and use a new kit. Contact the local Prestige Diagnostics representative.**

Procedure:

Reagent preparation:

Allow the reagents to reach room temperature (15 - 30°C). Check the Wash Buffer concentrate is free of salt crystals. If crystals have formed, re-solubilize by warming at 37°C, until crystals dissolve. Dilute the Wash Buffer (20X), for example by adding the 40 ml Wash Buffer concentrate to 760 ml of distilled or deionized water. Alternatively add a smaller quantity of concentrate to distilled water in the ratio 1/20. All other reagents are supplied as ready to use.

STEP 1

Preparation: Reserve 2 wells for Negative Control, 2 wells for Positive Control and one Blank (e.g. A1 – taking care that neither HRP conjugate or any samples are added to the Blank well). Note: If results are read using a plate reader having dual wavelength (450 / 600-650nm) then a Blank well need not be used. Assign samples to wells.

STEP 2

Addition of Sample Diluent and samples: Add 100 µl Positive Control and Negative Control to assigned wells (no Sample Diluent). Add 100 µl Sample Diluent to wells assigned for samples (not to blank well). Add 10 µl samples to their assigned wells (do not add anything to the Blank well).

STEP 3

Incubation: Cover the plate with a plate cover and incubate for 30 minutes at 37°C.

STEP 4

Washing: At the end of the incubation, remove the plate cover and discard the well contents by decantation or aspiration. Add 350 µl of diluted wash solution to all wells and soak for one minute before discarding the buffer. Repeat 4 more times for a total of 5 washes. Use of an automated microplate strip washer is recommended. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.

STEP 5

Addition of conjugate: Add 100 µl of Conjugate to each well except the Blank well and mix by tapping the plate gently.

STEP 6

Incubation: Cover the plate with plate cover and incubate for 20 minutes at 37°C.

STEP 7

Washing: At the end of the incubation, remove the plate cover and discard the well contents by decantation or aspiration. Add 350 µl of diluted wash solution to all wells and soak for one minute before discarding the buffer. Repeat 4 more times for a total of 5 washes. Use of an automated microplate strip washer is recommended. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.

STEP 8

Addition of the chromogens: Add 50 µl of Substrate Solution A and 50 µl of Substrate Solution B to each well including the blank. Incubate the plate at 37°C for 10 minutes. Ensure this incubation is carried out in the dark.

STEP 9

Stopping the Reaction: Add 50 µl of Stop Solution to each well and mix gently until the blue colour changes completely to yellow.

Measurement: Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm. Calculate the cut off value and determine the results. (Note: Absorbances must be read within 10 minutes of adding the Stop Solution).

Further Instructions for Washing:

To remove the potential for poor washing to cause false positive results and a high background, a 5 step automatic wash cycle is required with 350-400 µl of diluted wash buffer used per well per wash.
Ensure the microplate washer is adequately maintained. Ensure the liquid dispensing channels are not blocked or contaminated and that correct volume of wash buffer is dispensed each time into the wells.
If plates have to be washed manually, a process of 5 washing cycles, dispensing 350-400 µl/well, allowing 60 second soak and aspirating the liquid 5 times is recommended. If poor results are observed with high background, increase the number of washing cycles and soak time per well.
Capture all liquid waste aspirated and treat with 2.5% sodium hypochlorite for 24 hours before being disposed of in the appropriate way.

Calculation of results:

No matter how many plates are run at one time, the results of each one must be analysed individually. Results are calculated by relating each sample absorbance (Abs) to the cut off value (CO) calculated per plate. If absorbance readings are taken on single filter plate reader, the results should be calculated by subtracting the Blank well Abs from the absorbances of the samples and the controls. If absorbance readings are taken from a plate reader with dual filter capability, do not subtract the Blank well Abs value from the sample and control absorbances, just use the values obtained.

Calculation:

Cut off value (CO) = Negative Mean + 0.150.
Where Negative Mean = mean absorbance value for the 2 Negative Controls wells.
Important: If the mean Abs value of the Negative Controls is less than 0.050 then use 0.05 as the Negative Mean value.

Validation:

The assay results are only valid if the following criteria are met for each plate:
Blank well: the absorbance must be <0.080 at 450nm.
Positive Control: the absorbance must be ≥ 0.800 at 450/630nm or at 450nm after blanking.
Negative Control: the absorbance must be <0.100 at 450/630nm or at 450nm after blanking.
If one of the Negative Control absorbances does not match the above criteria, this value should be ignored and the Negative Mean value taken as the other Abs value. If both the Negative Control absorbance do not meet the criteria, the test is invalid and the whole run must be repeated.

Example:

Blank Value	A1: 0.015	450nm (blanking is required only when reading with a single filter)	
Negative control	0.022	0.024	
Positive control	2.381	2.407	

Calculation of Negative Mean: ((0.022 + 0.023/2) = 0.023
The calculated Negative Mean is less than 0.050, so use 0.05 as the Negative Mean value
Calculation of the cut off: 0.05 + 0.150 = 0.200

Interpretation of the results:

Negative Results: (Abs /CO <1) Any sample whose absorbance result is lower than the cut off value is considered negative. A negative result indicates that the sample is non-reactive for anti-SARS-CoV-2 IgG antibody and the patient is probably not infected with COVID-19.
Positive Results: (Abs /CO ≥ 1) Samples with absorbance results equal to or greater than the cut off value are regarded as initially reactive, indicating that anti-SARS-CoV-2 IgG antibody has potentially been detected. All initially reactive samples must be repeated in duplicate with this kit before final interpretation. Repeatedly reactive samples are regarded as positive for anti- SARS-CoV-2 IgG antibody and indicate probable current or previous infection with COVID-19.
Borderline: (Abs /CO = 0.9 – 1.1) Samples with absorbance to cut off ratio between 0.9 to 1.1 are considered borderline and retesting of these samples in duplicate is required to confirm the initial results.

Follow up, confirmation and supplementary testing of any positive sample with other analytical systems such as PCR is required. Clinical diagnosis should not be drawn using only the result of this ELISA test.
If, after re-testing of initially reactive samples, both wells give negative results (Abs/CO < 0.9), these samples should be considered negative and the original result must be classified as false positive. False positive results may occur due to several reasons, often associated with, but not limited to inadequate washing step.
If after retesting in duplicate, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive samples should be regarded as positive for anti- SARS-CoV-2 IgG.
After retesting in duplicate, samples with values close to the cut-off value should be interpreted with caution and considered as borderline, or uninterpretable at the time of sample taking.

Performance Characteristics:

Clinical Specificity: The clinical specificity of this assay was determined using a panel of samples from healthy donors. The clinical specificity, compared to another commercial COVID-19 IgG EIA, was 96.7%.
Clinical Sensitivity: Clinical sensitivity was assessed using a panel of samples, confirmed positive. The clinical sensitivity, compared to another commercial COVID-19 IgG EIA, was 98.3%.

Limitations:

- Positive results must be confirmed with another available method such as PCR and diagnosis made in conjunction with the other clinical and laboratory information.
- Antibody may be undetectable during the early stages of the disease. Negative results only indicate that the sample does not contain detectable levels of anti-SARS-CoV-2 and does not rule out the possibility of infection with the virus.
- Kit failure may result from using kits beyond the expiry date, poor washing procedures, contaminated reagents, improper operation of equipment, sample collection issues or timing errors.
- This kit is intended only for testing of serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled blood.
- This ELISA is a qualitative assay and the results cannot be used to measure antibody concentration.

References:

1. World Health Organisation Statement regarding cluster of pneumonia cases in Wuhan, China: 9 January 2020.
2. World Health Organisation. Coronavirus. www. who.int/health-topics /coronavirus.
3. Weiss SR, Leibowitz JL et al. Coronavirus pathogenesis. Adv Virus Res 2011; 81: 85-164.

REF	Catalogue number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by Date
	Manufacturer		

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