Human SARS-CoV-2 IgA Antibody ELISA Kit

Enzyme immunoassay kit for the quantification of SARS-CoV-2 IgA antibody in Human serum and plasma (heparin, citrate).

Catalog number: ARG83054

Package: 96 wells

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus that causes COVID-19 (coronavirus disease 2019), the respiratory illness responsible for the COVID-19 pandemic. Also colloquially known simply as the coronavirus, it was previously referred to by its provisional name, 2019 novel coronavirus (2019-nCoV), and has also been called human coronavirus 2019 (HCoV-19 or hCoV-19)

Each SARS-CoV-2 virion is 50–200 nanometres (2.0×10^{-6}–7.9×10^{-6} in) in diameter. Like other coronaviruses, SARS-CoV-2 has four structural proteins, known as the S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins; the N protein holds the RNA genome, and the S, E, and M proteins together create the viral envelope. Coronavirus S proteins are glycoproteins that are divided into two functional parts (S1 and S2). In SARS-CoV-2, the spike protein, which has been imaged at the atomic level using cryogenic electron microscopy, is the protein responsible for allowing the virus to attach to and fuse with the membrane of a host cell; specifically, its S1 subunit catalyzes attachment, the S2 subunit fusion [Wikipedia: Severe acute respiratory syndrome coronavirus 2].

PRINCIPLE OF THE ASSAY

This assay employs the qualitative determination enzyme immunoassay technique. SARS-CoV-2 nucleocapsid antigen has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any antibody present is bound by the immobilized antigen. After washing away any unbound substances, a Horseradish Peroxidase (HRP) labeled Protein conjugate is added to each microplate well and incubated. This conjugate binds to antigen-antibody complexes. After washing away any unbound reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of SARS-CoV-2 IgA bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	1 plate	4°C.
Control A (Negative Control)	2 ml (ready for use)	4°C
Control B (Cut-off Control)	3 ml (ready for use)	4°C
Control C (Positive Control)	2 ml (ready for use)	4°C
HRP-Streptavidin conjugate	20ml (ready for use)	4°C (Protect from light)
Sample Diluent Buffer	100 ml (ready for use)	4°C
20X Wash buffer	50 ml	4°C
TMB substrate	15 ml	4°C (Protect from light)
STOP solution	15 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 37°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using citrate or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. Diluted wash buffer is stable for 5 days at RT.
- Samples: Samples have to be diluted 1+100 with sample diluent. (e.g. Dispense 10µl sample and 1ml Sample Diluent Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.)

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of control and prepared samples into wells.
3. Cover wells and incubate for **60 minutes** at 37°C.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **100 µl** of HRP-Streptavidin conjugate into all well. Cover wells and incubate for **30 minutes** at RT in dark.
6. Aspirate each well and wash as step 4.
7. Add **100 µl** of TMB substrate into all well. Cover wells and incubate for **15 minutes** at RT in dark.
8. Add **100 µl** of Stop Solution to each well.
9. Read the OD with a microplate reader at **450/620 nm** immediately. It is recommended reading the absorbance within 30 min after adding Stop solution.

MEASUREMENT

1. Adjust the ELISA microwell plate reader to zero using the Substrate Blank. If- due to technical reasons- the ELISA microwell plate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results.
2. Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard/control and sample in the plate layout. Bichromatic measurement using a reference wavelength of 620 nm is recommended. Where applicable calculate the mean absorbance values of all duplicates.

RESULTS

1. Run Validation Criteria

   In order for an assay to be considered valid, the following criteria must be met:

   - Substrate Blank: Absorbance value < 0.100
   - Negative Control: Absorbance value < 0.200 and < Cut-off
   - Cut-off Control: Absorbance value 0.150 – 1.300
   - Positive Control: Absorbance value > Cut-off If these criteria are not met, the test is not valid and must be repeated.

2. Calculation of Results

   The Cut-off is the mean absorbance value of the Cut-off Control determinations. Example:

Absorbance value Cut-off Control (0.44) + absorbance value Cut-off control (0.42) = 0.86 / 2 = 0.43

Cut-off = 0.43

3. Results in Units [U]

[Units = U] = (Sample (mean) absorbance value x 10) / Cut-off

Example: (1.591 x 10) / 0.43 = 37 U

4. Interpretation of Results

- Positive: > 11 U

Antibodies against the pathogen are present.

There has been a contact with the antigen (pathogen resp. vaccine).

- Equivocal: 9-11 U

Antibodies against the pathogen could not be detected clearly.

It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as negative.

- Negative: < 9 U

The sample contains no antibodies against the pathogen.

A previous contact with the antigen (pathogen resp. vaccine) is unlikely.

Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data.

In immunocompromised patients and newborns serological data only have restricted value.

QUALITY ASSURANCE

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 7.2% and inter-assay precision was 6.3%.

Specificity

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 97.5% (97 % confidence interval: 95.58% - 99.85%)

Sensitivity

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 100.0 % (95% confidence interval: 47.82%- 100.0%).

Interferences

Interferences with 60 mg/ml Albumin, 0.4 mg/ml Bilirubin conjugated, 0.4 mg/ml Bilirubin unconjugated, 4 mg/ml Cholesterol, 10 mg/ml Hemoglobin and 15 mg/ml Triglycerides.

Cross Reactivity

Cross reaction with antibodies against Coronavirus HCoV-229E, Coronavirus HCoV-NL63, Adenovirus and Chlamydia pneumoniae cannot be excluded.