Human SARS-CoV-2 Spike Protein IgM ELISA Kit

Catalog No: abx365029

Size: 96T

Range: Qualitative

Sensitivity: Qualitative

Storage: Store at 2-8°C for 6 months.

Application: The qualitative detection of SARS-CoV-2 Spike Protein IgM in Human serum and plasma.

Principle of the Assay: This kit is based on indirect enzyme-linked immuno-sorbent assay technology. An antigen is pre-coated onto a 96-well plate. Controls, test samples and HRP-conjugated reagent are added to the wells and incubated. Unbound conjugates are removed using wash buffer. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient SARS-CoV-2 Spike Protein IgM will produce a blue coloured product, which then changes to yellow after adding the acidic stop solution. The intensity of the yellow colour is proportional to the SARS-CoV-2 Spike Protein IgM amount bound on the plate. The Optical Density (OD) is measured spectrophotometrically at 450 nm in a microplate reader, from which the presence of SARS-CoV-2 Nucleocapsid Protein IgM can be determined.

Kit Components

- Pre-coated 96-Well Microplate: 12 x 8
- Positive Control: 2 tubes
- Negative Control: 2 tubes
- Sample/Control Diluent Buffer: 20 ml
- Wash Buffer: (25X) 30 ml
- Detection Reagent: (100X) 120 µl
- Detection Reagent Diluent: 12 ml
- TMB Substrate: 10 ml
- Stop Solution: 10 ml
- Plate Sealer: 5
- Hermetic Bag: 1

Materials Required But Not Provided

- 37°C incubator
- Multi and single channel pipettes and sterile pipette tips
- Squirt bottle or automated microplate washer
- 1.5 ml tubes
- Distilled water
- Absorbent filter papers
- 100 ml and 1 liter graduated cylinders
- Microplate reader (wavelength: 450 nm, or dual-wavelength: 450/630 nm)
- ELISA Shaker

Abbexa Ltd, Cambridge, UK · Phone: +44 1223 755950 · Fax: +44 1223 755951
Abbexa LLC, Houston, TX, USA · Phone: +1 832 327 7413
Abbexa BV, Leiden, NL
www.abbexa.com · Email: info@abbexa.com

Protocol

A. Sample Preparation

Analyse immediately or store samples at 2-8°C (within 24 hrs). For long term storage, aliquot and store at -20°C or -80°C. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 2 hr. Centrifuge at approximately 1000 × g for 20 mins. If precipitate appears, centrifuge again. Assay immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 mins at 1000 x g, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples.

Notes:

- **Analyse serum/plasma samples at a 100-fold dilution (i.e add 10 µl of sample to 990 µl of Sample/Control Diluent Buffer).** Store frozen samples undiluted. Once ready to analyse, thaw samples and dilute .
- Fresh samples, or recently obtained samples, are recommended to prevent protein degradation and denaturation that may lead to erroneous results. Tubes for blood collection should be non-endotoxin.
- NaN_3 cannot be used as a test sample preservative, since it inhibits HRP.
- If a sample is not indicated in the manuals applications, a preliminary experiment to determine the suitability of the kit will be
- required.

B. Reagent Preparation

Wash Buffer: Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water). If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

Detection Reagent Working Solution Preparation:

1. Calculate the total volume of working solution required.
2. Dilute Detection Reagent 100-fold with Detection Reagent Diluent (i.e add 10 µl of Detection Reagent to 990 µl of Detection Reagent Diluent Buffer), and mix thoroughly. Pipette with a slow, smooth action to reduce volume errors.

Positive and Negative Control Working Solution Preparation:

1. Centrifuge the Control tubes at approximately 10,000 × g for 1 min.
2. Add 0.5 ml of Sample/Control Diluent Buffer to each Postive and Negative Control tube. Leave the tubes to stand for 10 mins, and gently invert several times to mix the solution.
3. Mix thoroughly with a pipette before use.

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C. Assay Protocol

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to measure in duplicate. Prepare all reagents as described above.

1. Set 2 positive and 2 negative control, test sample and control (zero) wells on the pre-coated plate respectively, and record their positions. *Add the solution to the bottom of each well without touching the side walls. Pipette the standards and samples up and down to mix before adding to the wells. Avoid foaming or bubbles.*
2. Aliquot 100 µl of negative and 100 µl positive control into the set wells. Leave one well as the control (zero) well. Please note that only TMB substrate and stop solution should be added to the control well.
3. Aliquot 100 µl of appropriately diluted sample (100-fold) into the test sample wells. Gently tap the plate to mix, or use a microplate shaker.
4. Cover the plate with a plate sealer and incubate for 45 mins at 37°C.
5. Remove the cover and discard the liquid. Wash the plate 3 times with Wash Buffer. *Fill each well completely with Wash buffer (350 µl) using a multi-channel Pipette or autowasher (1-2 mins soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.*
6. Aliquot 100 µl of Detection Reagent working solution to each well (except the blank well). Cover the plate with a plate sealer and incubate for 30 mins at 37°C.
7. Remove the cover, discard the solution, and repeat the wash process described in Step 5, 5 times.
8. Aliquot 90 µl of TMB Substrate into each well (including the control well). Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 15 mins. The incubation time is for reference only, the optimal time should be determined by end user. Avoid exposure to light.
9. Aliquot 50 µl of Stop Solution into each well (including the control well). It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
10. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the OD at 450 nm immediately.

Data Analysis:

- Mean OD of the Positive Control should be > 0.60.
- Mean OD of the Negative Control should be ≤ 0.10.
- Mean OD of the Blank Well should be ≤ 0.08
- CUT OFF value = Negative Control + 0.13 (if the Mean OD of the Negative Control is < 0.05, calculate at 0.05; otherwise, calculate at the actual value)

If the Postive Control value is > 0.6, the Blank Control value is ≤ 0.08 and the Negative Control value is ≤ 0.10, the test is valid, otherwise, the test is invalid.

If OD of Samples < CUT OFF, the test samples are considered negative.

If OD of Samples ≥ CUT OFF, the test samples are considered positive.

Precautions:

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
- The TMB substrate should be used under sterile conditions, and light exposure should be minimised. Unused substrate should be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial.
- Please note that this kit is optimised for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.

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