# ab275299 – SARS-CoV-2 IgM ELISA Kit

For the semi-quantitative measurement of IgM antibodies to SARS-CoV2 in human serum or plasma.
For research use only - not intended for diagnostic use.

**For overview, typical data and additional information please visit:**
**http://www.abcam.com/ab275299**

**Storage and Stability:** Store kit at 2-8°C immediately upon receipt. *Do Not Freeze reagents.*

## Materials Supplied

| Item | Quantity | Storage Condition |
|---|---|---|
| 96 well Precoated SARS-CoV-2 S1-RBD plate | 1 | 2-8°C |
| Positive Control: Recombinant Human anti-RBD IgM (ready to use) | 1 vial – 0.3 mL | 2-8°C |
| Calibrator: Recombinant Human anti-RBD IgM antibody (ready to use) | 1 vial – 0.3 mL | 2-8°C |
| Negative Control: Dilute normal human serum (ready to use) | 1 vial – 0.3 mL | 2-8°C |
| Detection Antibody: Goat Anti-Human IgM-Fc antibody – HRP conjugated (ready to use) | 1 vial – 11 mL | 2-8°C |
| Assay Dilution Buffer (ready to use) | 1 vial – 60 mL | 2-8°C |
| TNT Wash Buffer packet for reconstitution | 1 packet | 2-8°C |
| One Component TMB substrate: TMB/Hydrogen Peroxide | 1 vial – 12 mL | 2-8°C |
| Stop solution (ready to use) | 1 vial – 12 mL | 2-8°C |
| Sealing tape | 3 sheets | 2-8°C |

## Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Deionized water.
Precision pipettors, with disposable plastic tips.
Polypropylene or polyethylene tubes or low protein binding 96-well plates to prepare samples. *Do not use polystyrene, polycarbonate or glass tubes*.
A container to prepare TNT Wash Buffer.
A 96-well plate washer. *An autoclavable plate washer is recommended, if available*.
Disposable reagent reservoirs.
A microtiter plate reader for measuring absorbance at 450 nm and 570 nm

## Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. *Do Not Freeze reagents.*

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**TNT Wash Buffer:** Prepare Wash Buffer by reconstituting TNT packet in 1L of deionized water. Mix well

## Sample Preparation

This ELISA assay can be used for human serum or plasma collected in Potassium EDTA, Sodium Citrate or Lithium Heparin.
All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing of infectious agents.
100 µl of diluted sample or standard is required per well.
It is recommended that samples be assayed in duplicate each time the assay is performed.
Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at or below -70°C. Avoid repeated freeze-thaw cycles when storing samples.
If particulates are present in samples, centrifuge prior to use.
If blood samples are clotted, grossly hemolyzed, lipemic, or the integrity of the sample is of concern, make a note on the Plate Template and interpret results with caution.

**Sample preparation**
Serum and plasma – Recommended dilution is 1:100. For example, add 5 µl of plasma/serum into 495 µl of 1X Dilution Buffer to give a 1:100 dilution. Mix thoroughly. *Upon adding sample to dilution buffer, a noticeable flocculence is common and expected.*

## Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate

Use a Plate Template to record the well locations of the Positive Control, Calibrator, Negative Control, and unknown samples.

1. Add 100 µl of controls and diluted samples to the appropriate wells. Run each control, and the samples in duplicate if appropriate.

2. Carefully cover the wells with a new sealing tape and incubate for 30 minutes at room temperature, 20-25°C.

3. Carefully remove the adhesive plate cover and wash 5 times with TNT Wash Buffer, as described in the Plate Washing section below.

4. Rinse the tips of the plate washer by dispensing the TNT Wash Buffer into the wash trough and aspirating the solution. Repeat this step 5 times. For automated plate washers, program the rinse step accordingly. ***Note***: This initial rinse step is necessary especially if the plate washer has been idle for several days or longer. Automated plate washers are susceptible to microbial growth in the fluid lines and cavities.

5. Aspirate the solutions from the wells. Fill the wells to about 90% (250 µl) full with TNT Wash Buffer and then aspirate the wash solution. Repeat this wash step 4 more times. For

automated plate washers, program 5 washes at 300 µl per wash, according to the manufacturer's instructions.

6. Add 100 µl of Detection Antibody to each well. Mix by gently tapping the plate several times.

7. Carefully attach a new adhesive plate cover and incubate the plate for 30 minutes at room temperature, 20-25°C.

8. Carefully remove the adhesive plate cover and wash 5 times with TNT Wash Buffer (see Plate Washing section above).

   *Do NOT use glass pipette to measure the TMB Substrate Solution. Do NOT cover the plate with aluminum foil or metalized mylar. If the solution is blue before use, DO NOT USE IT!*

9. Add 100 µl of TMB Substrate Solution into each well and incubate at room temperature (20-25°C) in the dark for 15 minutes. Do NOT cover plate with a plate sealer

10. Stop the reaction by adding 100 µl of Stop Solution to each well. Tap plate gently to mix. The solution in the wells should immediately change color from blue to yellow

11. Note: Wipe the underside of the wells with a lint-free tissue.

    Measure the absorbance on an ELISA plate reader set at 450 nm and 570 nm within 30 minutes of stopping the reaction.

## Calculations:

1. For each well, derive the corrected A450 value by subtracting the value measured at 570 nm from the value measured at 450 nm.
2. For calibrator, each control and each sample
   a. Calculate the mean of the corrected A450 values.
   b. Calculate the standard deviation (StdDev) of the corrected A450 values.
   c. Calculate the Percent Coefficient of Variation (%CV) of the corrected A450 values: %CV = StdDev ÷ mean × 100
3. Derive the ratio of the Positive Control to the Calibrator by dividing the mean of the corrected A450 values of the Positive Control by the mean of the corrected A450 values of the Calibrator.
4. Derive the ratio of the Negative Control to the Calibrator by dividing the mean of the corrected A450 values of the Negative Control by the mean of the corrected A450 values of the Calibrator.
5. For each sample, calculate the ratio for the sample by dividing the mean of the corrected A450 values of the sample by the mean of the corrected A450 values of the Calibrator.

## Validity and Acceptability:

The following conditions must be met for the assay to be valid and acceptable



1. %CV for Positive control is less than 15.
2. %CV for Calibrator is less than 15.
3. The ratio of the Positive Control is between 3 and 8.
4. The ratio of the Negative Control is less than 0.5

The following condition must be met for values for an individual sample to be valid and acceptable:
%CV for the sample is less than 15.

**If any condition is not met, repeat the assay**.

## Interpretation of the results:

Samples for which the ratio is 1.0 or greater are positive.
Samples for which the ratio is 0.9 or less are negative.
Samples for which the ratio is between 0.9 and 1.0 are equivocal requiring repeating of the test.
If the results of the subsequent assessment are also equivocal, no determination can be made.

## Limitations of the assay:

1. This test is for the semi-quantitative assessment of seroconversion. Higher absorbance values correlate with higher relative concentration of specific antibody. This assay detects antibody specific to the RBD protein only and does not reflect total antibody response to other SARS-CoV-2 associated proteins.

2. Microbial contamination of samples or reagents, cross contamination of samples and/or kit reagents or extreme temperature excursions may yield erroneous results.

3. Previous infection with SARS-CoV-1 may result in a positive test result.

**Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:**
www.abcam.com/protocols/the-complete-elisa-guide

**For technical support contact information, visit:** www.abcam.com/contactus