Human SARS-CoV-2 Spike (Trimer) IgG ELISA Kit

Catalog Number: ATK00022

MATERIALS PROVIDED & STORAGE CONDITIONS

PART	Format	Description	STORAGE
Capture Plate	1 plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Spike (Trimer) protein.	Store in sealed at - 20℃.
Positive Control	1 vial	10μL/vial, 0.8mg/mL Recombinant humanized antibody specific for Spike (Trimer) protein with preservatives.	Store at - 20℃.
Detection A	1 vial	10μL/vial biotin labeled anti-human IgG antibody, 1:10000 diluted by dilution buffer before used.	Store at - 20℃.
Detection B	1 vial	12μL/vial HRP labeled Streptavidin(SA), 1:2000 diluted by dilution buffer before used.	Store at - 20℃.
Sample Dilution Buffer	1 bottle	25 ml/bottle diluent (including preservative) was used to dilute the Positive Control, Serum and Plasma.	Store at 4℃.
Reagent Dilution Buffer	1 bottle	25 ml/bottle diluent (including preservative) was used to dilute the Detection A and Detection B.	Store at 4℃.
Wash Buffer Concentrate	1 bottle	25 mL/bottle of a 20-fold concentrated solution of buffered surfactant with preservative. 1:20 diluted by deionized water before used.	Store at 4℃.
Color Reagent TMB	1 bottle	12 mL/ bottle of TMB(tetramethylbenzidine) .	Store at 4℃.
Stop Solution	1 bottle	6 mL/ bottle.	Store at 4℃.
Plate Sealer	4 strips	Adhesive strips.	RT.

* Provided this is within the expiration date of the kit.

INTENDED USE

Used for the quantitative determination of Spike (Trimer) specific IgG antibody concentration in serum and plasma.

PRINCIPLE OF THE ASSAY

This kit employs the quantitative indirect enzyme immunoassay technique. A recombinant Spike (Trimer) protein has been pre-coated onto a microplate. Positive Control and samples are pipetted into the wells and the Spike (Trimer) specific antibody will be captured by immobilized Spike (Trimer) protein. After washing away any unbound substances, a biotin-conjugated anti-human IgG antibody is added to the wells. Next, adding HRP labeled Streptavidin(SA) into the well and then removing any unbound antibody-enzyme reagent by washing, a Color Reagent TMB is added to the wells and color develops in proportion to the concentration of Spike (Trimer) specific IgG antibody in the sample.

OTHER SUPPLIES REQUIRED

• Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.

• Pipettes and pipette tips.

• Deionized or distilled water.

• 500 mL graduated cylinder.

• Squirt bottle, manifold dispenser, or automated microplate washer.

• Test tubes for dilution of standards.

SAMPLE COLLECTION & STORAGE

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

Handle all blood and serum as if capable of transmitting infectious agents. The NCCLS provides recommendations for handling and storing serum and plasma specimens (Approved Standard-Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).

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

Plasma - Collect plasma using EDTA, heparin, or citrate 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℃. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

20-fold Wash Buffer Concentrate: If there is crystal precipitation in the Wash Buffer Concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 25 mL of Wash Buffer Concentrate to 475 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Positive Control: Gently shake and mix before used. Centrifuge instantaneously with palm centrifuge to make the liquid at the bottom of the tube. The concentration of positive control is 0.8mg/ml, dilute the Positive Control 1:100 to stock solution of standard at a concentration of 8ug/ml with Sample Dilution Buffer first, and then the stock solution of standard was diluted with sample diluent 1:1000 to get the first standard point of 8000pg/mL. Please prepare 7 tubes containing 0.5mL Sample Diluent and produce a double dilution serie to obtain 7 points of diluted standard such as 8000pg/mL, 4000pg/mL, 2000pg /mL, 1000pg/mL, 500pg /mL, 250pg /mL, 125pg /mL, and the last EP tubes with Sample Diluent is the blank as 0pg/mL.

Sample Preparation: Dilute test samples with Sample Dilution Buffer with a volume ratio of 1:1000~1:5000. For example, dilute 10 μL of sample with 900 μL of Sample Dilution Buffer.

Detection A(Biotin labeled): Shake and mix before used. Centrifuge instantaneously with palm centrifuge to make the liquid at the bottom of the tube. Dilute the Detection A 1:10000 to the working concentration with Reagent Dilution Buffer.

Detection B(HRP labeled): Shake and mix before used. Centrifuge instantaneously with palm centrifuge to make the liquid at the bottom of the tube. Dilute the Detection A 1:2000 to the working concentration with Reagent Dilution Buffer.

Color Reagent TMB: Protect from light, Add 100μL of Color Reagent TMB to each well. Incubate for 8~10 minutes at room temperature.

Stop solution: 50μL/well after color development.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal, store at -20℃.
3. In separate wells, add 100ul of diluted Positive Control and the diluted Samples. Cover the plate with Plate Sealer and incubate 30mins at 37℃.
4. Aspirate each well and wash, repeating the process three times. Wash by filling each well with Wash Buffer (300 μ L) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 μL of diluted Detection A(Biotin labeled) solution to each well. Cover the plate with Plate Sealer and incubate 30mins at 37℃.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 μL of diluted Detection B(HRP labeled) solution to each well. Cover the plate with Plate Sealer and incubate 30mins at 37℃.

8. Repeat the aspiration/wash as in step 4.

9. Add 100μL of Color Reagent TMB to each well. Incubate for 8~10 minutes at 37℃. Protect from light.

10. Add 50μL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

11. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Draw a best fit curve by plotting the known concentration of the Positive control (X-axis) against the O.D. value of the Positive control (Y-axis), and then calculate the target antibody concentration in the sample by the regression equation of the standard curve.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Detection Range

125pg/mL—8000pg/mL

SENSITIVITY

The minimum detectable dose (MDD) of Spike (Trimer) IgG antibody is typically less than 100pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

Precision

Intra-Assay Precision (Precision within an assay): 12%

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays): <15%

Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision.

Stability

When the kit was stored at the recommended temperature for 6 months, the signal intensity decreased by less than 10%.