AtaGenix

DATASHEET *Version 2.5*

SARS-CoV-2(2019-nCoV) Surrogate Virus Neutralization Quantitative Test Kit

Catalog Number: ATK00021

MATERIALS PROVIDED & STORAGE CONDITIONS

PART	Format	Description	STORAGE
Capture Plate	1 plate	96 well polystyrene microplate (12 strips of 8 wells) coated with RBD protein.	Store in sealed at - 20℃.
Positive Control	1 vial	12μL/vial, 1mg/mL antibody specific for RBD protein with preservatives.	Store at - 20℃.
Negative Control	1 vial	60μL/vial, negative control with preservatives, 1:10 diluted by dilution buffer before used.	Store at - 20℃.
Detection A	1 vial	12μL/vial HRP labeled ACE2 protein (including preservative) , 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 ,Negative 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.	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.**

FUNCTION

Determine the concentration of RBD neutralizing antibody in serum and plasma samples.

PRINCIPLE OF THE ASSAY

RBD protein has been pre-coated onto a microplate, Standards and samples are pipetted into the wells, Then a HRP-labeled protein ACE2 is added to the wells, Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops inverse proportion to the concentration of RBD Neutralization antibody in the initial step.

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.

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.

Sample Preparation: Dilute test samples with **Sample Dilution Buffer** with a volume ratio of 1:9. For example, dilute 10 µL of sample with 90 µL of Sample Dilution Buffer

Positive Control: shake and mix before used. Centrifuge instantaneously with palm centrifuge to make the liquid at the bottom of the tube. Use **Sample Dilution Buffer 100 times** dilution the **Positive Control** then obtain the first standard point of 10ug/mL, the multiple proportion dilution of the standard was selected, the concentration of the 7 standard sample were 10ug /mL, 5ug /mL, 2.5ug /mL, 1.25ug /mL, 0.625ug /mL, 0.3125ug /mL, 0.15625ug /mL respectively.

Negative Control: shake and mix before used. Centrifuge instantaneously with palm centrifuge to make the liquid at the bottom of the tube. Dilute the **Negative Control** 1:10 to the working concentration with **Sample Dilution Buffer.**

Detection A(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.

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.

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.

3. In separate wells,add 50ul of diluted **Standard**, diluted **Negative Control**, or the diluted **Samples.**Cover the plate with **Plate Sealer** and incubate 30mins at 37℃.

4. Add 50 µL of diluted **Detection A** solution to each well.Set the plate on the microplate mixer with shaking 5mins.

5. Cover the plate with **Plate Sealer** and incubate 1 hour at 37℃.

6. Aspirate each well and wash, repeating the process three times. Wash by filling each well with Wash Buffer (300µL) 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.

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

8. 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.

9. 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, Negative Control, and Samples optical density (O.D.). Then the inhibition rate is calculated as follows:

$$\text{Inhibition} = \left(1 - \frac{\text{OD value of Sample}}{\text{OD value of Negative Control}}\right) * 100\%$$

Create a standard curve by reducing the data using computer software capable. Construct a standard curve by plotting the each standard inhibition rate on the Y-axis against the standard concentration on the X-axis and draw a best fit curve through the points on the graph. 4-5 points with inhibition rate between 10% - 90% were selected to draw the standard curve.

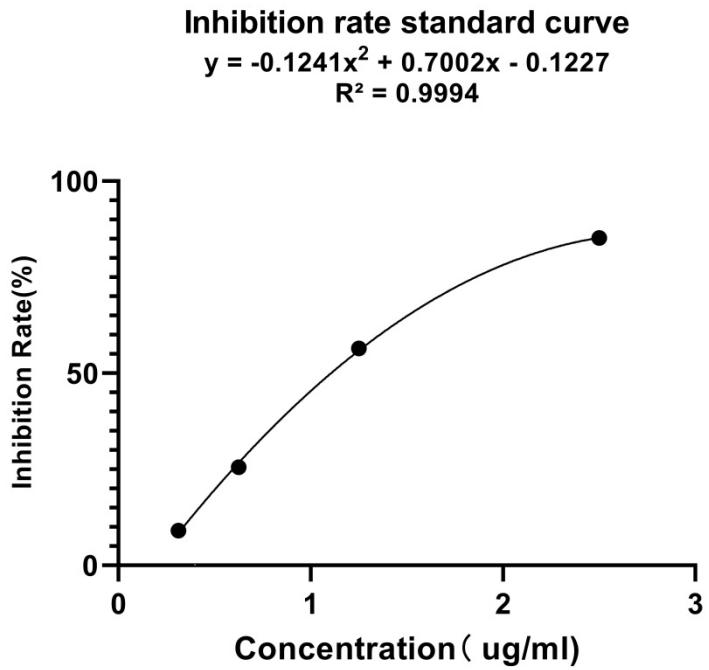
Then the inhibition rate of the sample is substituted into the regression equation of the standard curve to calculate the concentration, The concentration calculated on the standard curve must be multiplied by the dilution multiple to calculate the actual concentration of RBD neutralizing antibody.

If the sample test value is beyond the standard curve, the dilution ratio can be adjusted appropriately and re determined, or the antibody concentration in the sample can be estimated, and several gradients can be pre-diluted before the test.

TYPICAL DATA

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Standard Concentration（ug/mL）	O.D.	Inhibition Rate
10	0.06	97.08%
5	0.11	94.41%
2.5	0.29	85.18%
1.25	0.84	56.44%
0.625	1.44	25.53%
0.3125	1.76	9.04%
0.15625	1.72	10.96%
Negative Control	1.94	/



LINEARITY

0.15625ug/mL—10ug/mL

Precision

Intra-Assay Precision (Precision within an assay): <8%

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

Inter-Assay Precision (Precision between assays): <10%

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%.