

MATERIALS PROVIDED & STORAGE CONDITIONS

PART	Format	Description	STORAGE
Capture Plate	1 plate	96 well polystyrene microplate (12 strips of 8 wells) coated with RBD(K417N, L452R, T478K) protein.	Store in sealed at - 20°C.
Positive Control	1 vial	12µL/vial, 1mg/mL antibody specific for RBD protein with preservatives, 1:100 diluted by dilution buffer before used. Final concentration was 10µg/mL.	Store at - 20°C.
Negative Control	1 vial	60µL/vial, negative control with preservatives, 1:10 diluted by dilution buffer before used.	Store at - 20°C.
Detection A	1 vial	12µL/vial HRP labeled ACE2 protein (including preservative) , 1:2000 diluted by dilution buffer before used.	Store at - 20°C.
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°C.
Reagent Dilution Buffer	1 bottle	25 ml/bottle diluent (including preservative) was used to dilute the Detection A.	Store at 4°C.
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°C.
Color Reagent TMB	1 bottle	12 mL/ bottle of TMB(tetramethylbenzidine) .	Store at 4°C.
Stop Solution	1 bottle	6 mL/ bottle.	Store at 4°C.
Plate Sealer	4 strips	Adhesive strips.	RT.

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

SPECIFICITY

SARS-CoV-2 Surrogate Virus Neutralization Test Kit(K417N, L452R, T478K) can detect circulating neutralizing antibodies against SARS-CoV-2 that block the interaction between the receptor binding domain of the viral spike glycoprotein RBD(K417N, L452R, T478K) with the ACE2 cell surface receptor. The assay detects any antibodies in serum and plasma that neutralize the RBD(K417N, L452R, T478K)-ACE2 interaction. The test is both species and isotype independent.

PRINCIPLE OF THE ASSAY

SARS-CoV-2 Surrogate Virus Neutralization Test Kit(K417N, L452R, T478K) is a blocking ELISA detection tool, which mimics the virus neutralization process. The kit contains two key components: the HRP labeled recombinant human ACE2 and the SARS-CoV-2 RBD(K417N, L452R, T478K)protein . The protein-protein interaction between RBD(K417N, L452R, T478K) and ACE2-HRP can be blocked by neutralizing antibodies against SARS-CoV-2 RBD(K417N, L452R, T478K).

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 μ L of sample with 90 μ L 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. Dilute the Positive Control 1:100 to the working concentration with Sample Dilution Buffer.

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 100 μ L of Color Reagent TMB to each well. Incubate for 8~10 minutes at room temperature.

Stop solution: 50 μ L/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 $\leq -20^{\circ}\text{C}$. 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 $\leq -20^{\circ}\text{C}$. 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 50 μ L of diluted Positive Control, diluted Negative Control, or the diluted Samples. Cover the plate with Plate Sealer and incubate 30 mins at 37°C.
4. Add 50 μ L of diluted Detection A solution to each well. Set the plate on the microplate mixer with shaking 5 mins.
5. Cover the plate with Plate Sealer and incubate 1 hour at 37°C.
6. 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.
7. Add 100 μ L of Color Reagent TMB to each well. Incubate for 8~10 minutes at 37°C. Protect from light.
8. Add 50 μ L 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.

INTERPRETATION OF RESULTS

To assure the validity of the results, each assay must include both Positive and Negative Controls. The net optical density (OD450) of each control must fall within the ranges listed in the following table. If OD450 values of controls do not meet the requirements in the following table, the test is invalid and must be repeated.

Items	OD450 value	Control Result for Valid Assay
Positive Control	< 0.3	Passed
Negative Control	> 0.9	Passed

The positive cutoff and negative cutoff for SARS-CoV-2 neutralizing antibody detection can be used for interpretation of the inhibition rate. The operator can determine the result of the sample by comparing the inhibition rate to the following table.

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

Cutoff Interpretation

Items	Cutoff	Result	Interpretation
Neutralizing antibody test	≥ 20%	Positive	Neutralizing antibody detected
	< 20%	Negative	No detectable neutralizing antibody

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%.