

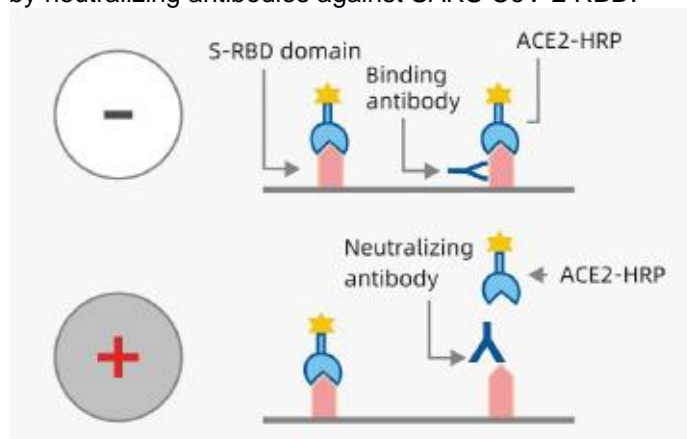
SARS-CoV-2(2019-nCoV) Surrogate Virus Neutralization Test Kit

Catalog No: CKV001

Size: 1 x 96 tests

Specificity: SARS-CoV-2(2019-nCoV) Surrogate Virus Neutralization Test Kit can detect circulating neutralizing antibodies against SARS-CoV-2 that block the interaction between the receptor binding domain of the viral spike glycoprotein (RBD) with the cell surface receptor ACE2. The assay detects any antibodies in serum and plasma that neutralize the RBD-ACE2 interaction. The test is both species and isotype independent.

Assay Principle: The SARS-CoV-2(2019-nCoV) Surrogate Virus Neutralization Test Kit 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 human RBD protein. The protein-protein interaction between RBD and ACE2-HRP can be blocked by neutralizing antibodies against SARS CoV-2 RBD.



Reagents Provided:

Component	Description	Quantity	Storage
CKV001-P	96-well microplate (12 strips of 8 wells) coated with RBD protein	1 plate	-20°C
CKV001-A	Wash Buffer Concentrate (20x) contains preservative	1 bottle, 25 ml	4°C
CKV001-B	Positive Control (100x), 1 mg/ml antibody specific for RBD protein, with preservative.	1 vial, 30 µl	-20°C
CKV001-C	Negative Control (100x), with preservative.	1 vial, 30 µl	-20°C
CKV001-D	Detection A: HRP-labeled ACE2 protein, with preservative	1 vial, 13 µl	-20°C
CKV001-E	Sample Dilution Buffer, used to dilute Positive, Negative Controls	1 bottle, 25 ml	4°C
CKV001-F	Reagent Dilution Buffer, used to dilute Detection A	1 bottle, 25 ml	4°C
CKV001-G	TMB Color Reagent (tetramethylbenzidine)	1 bottle, 12 ml	4°C
CKV001-H	Stop Solution	1 bottle, 6 ml	4°C
CKV001-S	Plate sealers	4 seals	RT



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- Reagents and Equipment Required:**
- Pipettes covering 0-10 μ l and 200-1000 μ l, and tips
 - 12-channel pipette covering 30-300 μ l
 - Paper towels or laboratory wipes
 - Polypropylene conical 50 ml tubes, 1.5 ml flip-cap tubes
 - 500 ml graduated cylinder
 - Deionized or distilled water
 - Microplate reader measuring at 450 nm, with correction wavelength at 540 nm or 570 nm
 - Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm
 - Automatic plate washer or wash bottle

Warnings: **Warning** – Avoid skin and eye contact when using TMB substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

- Precautions:**
- **DO NOT** mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
 - **DO NOT** pipette reagents by mouth.
 - Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
 - Keep plate covered except when adding reagents, washing, or reading.
 - **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

Reagent Preparation: **Bring all reagents to room temperature before use.**

1. **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.
2. **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
3. **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**.
4. **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** 1:100 to the working concentration with **Sample Dilution Buffer**.
5. **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**.
6. **Color Reagent TMB:** Protect from light, Add 100 μ L of **Color Reagent TMB** to each well. Incubate for 10 minutes at room temperature.
7. **Stop solution:** 50 μ L/well after color development.



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Samples: 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 1000xg. 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 1000xg 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.**

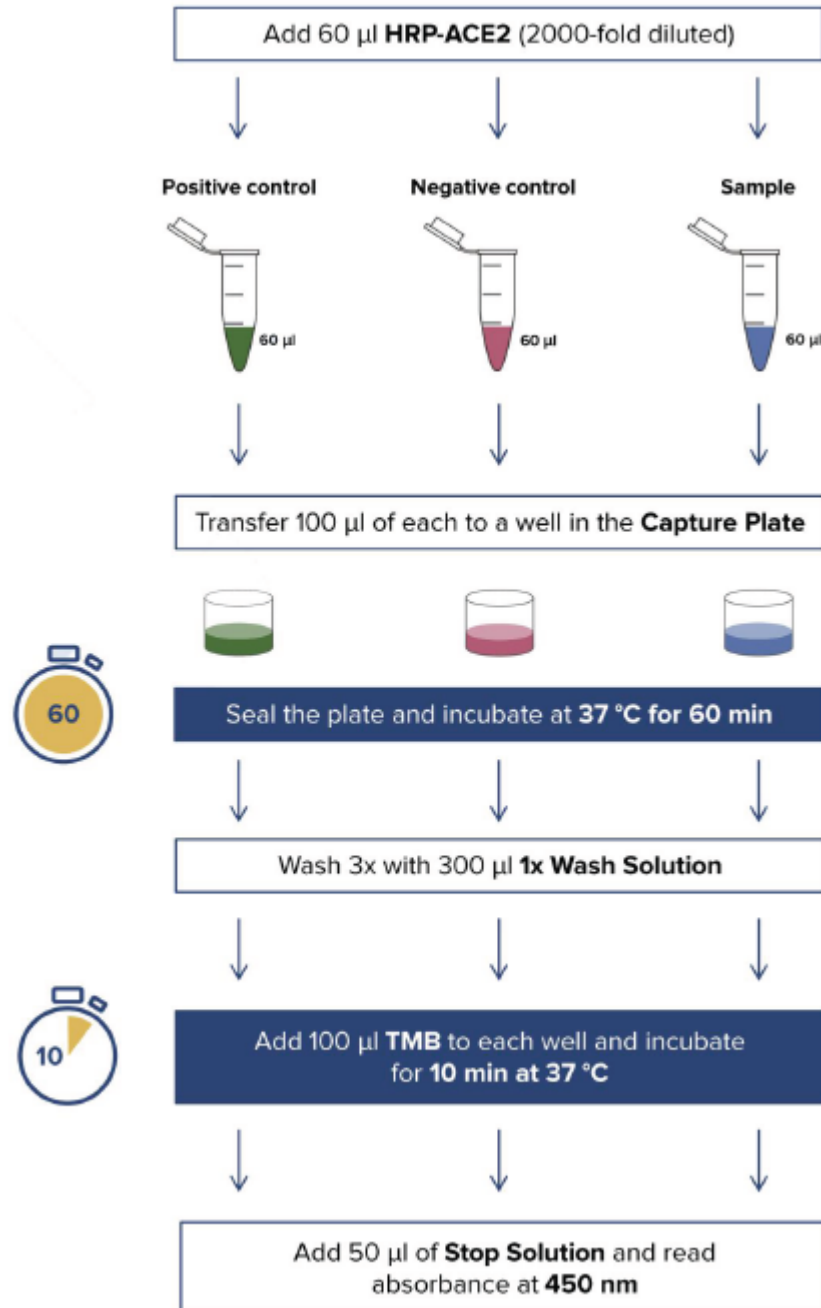
It is recommended that all standards, controls, and samples be assayed in duplicate.

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 tubes, mix the diluted **Positive Control**, diluted **Negative Control**, or the **Samples** with the diluted **Detection A** solution with a volume ratio of 1:1. For example, mix 100 μL **Positive Control** with 100 μL **Detection A** solution. This step may be done in clean, uncoated 96-well ELISA plates.
4. Add 100 μL each of the **Positive Control** mixture, the **Negative Control** mixture, and the **Sample** mixture to the corresponding wells. Cover the plate with **Plate Sealer** and incubate 1 hour at 37°C .
5. Aspirate each well and wash, repeating the process three times. Wash by filling each well with **Wash Buffer** (300 μL) using a squirt bottle, manifold dispenser, or automatic washer. 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.
6. Add 100 μL of **Color Reagent TMB** to each well. Incubate for **10** minutes at 37°C . Protect from light.
7. Add 50 μL 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.
8. 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.



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ASSAY FLOWCHART



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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.

Item	OD ₄₅₀ 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.

$$\text{Inhibition} = [1 - (\text{OD value of Sample} / \text{OD value of Negative Control})] \times 100\%$$

Cutoff Interpretation:

Item	Cutoff	Result	Interpretation
Neutralizing Antibody Test	$\geq 20\%$	Positive	Neutralizing Ab
	$\leq 30\%$	Negative	No neutralizing Ab

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.

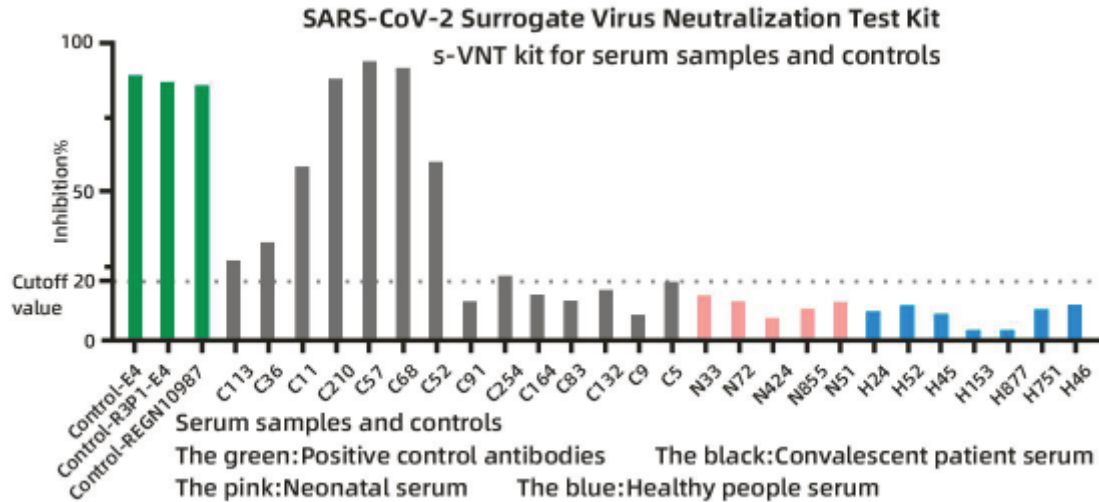
Stability:

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



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Serum Validation Data



Disclaimer:

This information is believed to be correct but does not claim to be all-inclusive and should be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling or from contact with the above product.

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