# Anti-SARS-CoV-2 neutralizing antibody titer serologic assay kit

**Pack Size: 96 tests / 480 tests**

**Catalog Number：** TAS-K003

**IMPORTANT: Please carefully read this manual before performing your experiment.**

***For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedures***

## PRINCIPLE OF THE ASSAY

The newly identified 2019 novel coronavirus (SARS-CoV-2) has posed a serious threat to human health.. It is helpful to develop the Anti SARS-CoV-2 neutralizing antibody titer serologic assay kit to test the level of protective neutralizing antibody in serum.

This assay kit employs a competitive ELISA format and is uniquely suitable for rapid high-throughput detection of the titer of Anti-SARS-CoV-2 neutralizing antibody in serum / plasma. Briefly, we provide you with a biotinylated Human ACE2 protein, a SARS-CoV-2 Spike protein RBD, an Anti-SARS-CoV-2 neutralizing antibody (human IgG1) (as Reference), and Streptavidin-HRP reagent.

Your experiment will include 4 simple steps:

a) Coat the plate with SARS-CoV-2 Spike protein RBD.

b) Add 50ul biotinylated human ACE2 to coated plate.

c) Add the reference in kit or your samples from serum or plasma of interest.

d) Add Streptavidin-HRP followed by TMB or other colorimetric HRP substrate.

Finally, the titer of your samples to inhibit Spike protein RBD: ACE2 binding will be determined by comparing OD readings among different experimental groups.



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## MATERIALS PROVIDED

**TABLE 1. MATERIALS PROVIDED**

| Catalog | Components | Size (96 tests) | Size (480 tests) | Format | Storage |
|---|---|---|---|---|---|
| TAS003-C01 | SARS-CoV-2 S protein RBD | 10 | 60 | Powder | -20℃ |
| TAS003-C02 | Biotinylated Human ACE2 | 10 | 10 | Powder | -20℃ |
| A003-214 | Streptavidin-HRP | 10 | 10 | Powder | -20℃, avoid light |
| SAD-S35 | Anti-SARS-COV-2 neutralizing antibody | 20 | 50 | Powder | -20℃ |

## RECONSTITUTION

Reconstitute the provided lyophilized materials to stock solutions with PBS as recommended in **Table 2.1 and Table 2.2,** Solubilize for 15 to 30 minutes at room temperature with occasional gentle mixing. **Avoid vigorous shaking or vortexing**.

The reconstituted stock solutions should be stored at -70℃. **It is recommended not to freeze thaw more than 3 times.**

To avoid surface adsorption loss and inactivation, the reconstituted protein must NOT be aliquoted to less than 5 μg per vial.

***Note**: Streptavidin-HRP stock solution should be protected from light.*

**TABLE 2.1. RECONSTITUTION METHODS FOR 96 TESTS**

| Catalog | Components | Size | Stock Solution Con. | Reconstitution Buffer and Vol. |
|---|---|---|---|---|
| TAS003-C01 | SARS-CoV-2 S protein RBD | 10 | 100 μg/mL | 100 μL water |
| TAS003-C02 | Biotinylated Human ACE2 | 10 | 100 μg/mL | 100 μL water |
| A003-214 | Streptavidin-HRP | 10 | 50 μg/mL | 200 μL water |
| SAD-S35 | Anti-SARS-COV-2 neutralizing antibody | 20 | 200 μg/mL | 100 μL water |

**TABLE 2.2. RECONSTITUTION METHODS FOR 480 TESTS**

| Catalog | Components | Size | Stock Solution Con. | Reconstitution Buffer and Vol. |
|---|---|---|---|---|
| TAS003-C01 | SARS-CoV-2 S protein RBD | 60 | 100 μg/mL | 600 μL water |
| TAS003-C02 | Biotinylated Human ACE2 | 10 | 100 μg/mL | 100 μL water |
| A003-214 | Streptavidin-HRP | 10 | 50 μg/mL | 200 μL water |
| SAD-S35 | Anti-SARS-COV-2 neutralizing antibody | 50 | 200 μg/mL | 250 μL water |



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## SHIPPING AND STORAGE

**All components are shipped in lyophilized state at room temperature.** This product is stable after storage at:

1) Room temperature (RT) for 1 month in lyophilized state.
2) -20℃ for 1 year in lyophilized state.
3) -70℃ for 6 months under sterile conditions after reconstitution.

## MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED (for 96 tests)

**Coating Buffer** 15 mmol/L sodium carbonate ($Na_2CO_3$), 35 mmol/L sodium hydrogen carbonate ($NaHCO_3$), 7.7 mmol/L sodium azide ($NaN_3$), pH 9.6, 12 mL is sufficient for 96 tests.

**Wash Buffer** TBS with 0.05% (v/v) Tween-20 (pH7.4), 500 mL is sufficient for 96 tests.

**Blocking Buffer** **Wash Buffer** with 2% (w/v) bovine serum albumin (BSA) *(Sigma-Aldrich, Catalog # A4737),* 35 mL is sufficient for 96 tests.

**Dilution Buffer** **Wash Buffer** with 0.5% (w/v) bovine serum albumin (BSA) *(Sigma-Aldrich, Catalog # A4737),* 50 mL is sufficient for 96 tests.

**Substrate Dilution Buffer** 50 mM disodium hydrogen phosphate ($Na_2HPO_4$) and 25 mM citric acid, adjust pH to 5.5 with 1 M Sodium hydroxide (NaOH), 25 mL is sufficient for 96 tests.

**Substrate Stock Solution** 10 mg/mL TMB *(Sigma-Aldrich, Catalog # 860336)* in Dimethyl sulfoxide *(Sigma-Aldrich, Catalog # D8418)*, 1 mL is sufficient for 96 tests. **Protect from light**.

**TMB Substrate Working Solution**

For **each plate,** dilute 250 µL substrate stock solution in 25 mL substrate dilution buffer and add 12 µL 5% $H_2O_2$ (pipette 10 µL 30% $H_2O_2$ into 50 µL distilled water), mix well.

***Notes:***

1) *The TMB Substrate Working Solution should be freshly prepared and used within 15 minutes.*
2) *If you choose to use other commercially available ready-to-use TMB substrate solutions, you should follow the manufacturer's instruction.*

**Stop Solution** 1 M sulfuric acid (aqueous), 6 mL is sufficient for 96 tests.

**High binding surface 96-well microplate, clear flat bottom** *(Corning, Catalog # 42592)*

**Microplate sealing film** *(Sigma-Aldrich, Catalog # Z724742)*

**Pipettes and pipette tips**

**UV/Vis microplate spectrophotometer** (absorbance 450 nm, correction wavelength set to 630 nm).



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## RECOMMENDED PROTOCOL

**1. Preparation**

Reconstitute and store all reagents as recommended.

**2. Coating**

1) Dilute **SARS-CoV-2 S protein RBD** stock solution (100 μg/mL) to 0.5 μg/mL with **Coating Buffer** to make **SARS-CoV-2 S protein RBD** working solution.
2) Please leave two wells uncoated for **No-Coating Control (Table 3)**.
3) Add 100 μL of **SARS-CoV-2 S protein RBD** working solution (0.5 μg/mL) to each well, seal the plate with microplate sealing film and incubate overnight (or 16 hours) at 4℃.

**3. Washing**

Remove the remaining solution by aspiration, add 300 μL of **Wash buffer** to each well, gently tap the plate for 1 minute, remove any remaining **Wash Buffer** by aspirating or decanting, invert the plate and blot it against paper towels. **Repeat the wash step above for three times**.

**4. Blocking**

Add 300 μL **Blocking Buffer** to each well, seal the plate with microplate sealing film and incubate at 37℃ for 1.5 hours.

**5. Washing**

Repeat step 3. At meantime, you can start to prepare your **samples**.

**6. Binding**

1) Dilute **Biotinylated Human ACE2** stock solution (100 μg/mL) to 0.12 μg/mL with **Dilution Buffer** to make **Biotinylated Human ACE2** working solution.
2) For **No-Binding Control** wells, please add 50 μL **Dilution Buffer**.
3) For all other wells, please add 50 μL **Biotinylated Human ACE2** working solution.

**7. Add Samples**

1) Make series dilution of the samples as appropriate.
2) If you intend to use the provided **Anti-SARS-COV-2 neutralizing antibody** as a reference (Ref.), you may dilute the inhibitor as recommended in **Figure 3**. And plate layout as recommended in **Figure 4**.
3) For **Positive control** wells, please add 50 μL Dilution Buffer.
4) For all other wells, Add 50 μL of sample solution to each well according to our recommendation (**Figure 1**) or your own plate setup.
5) Seal the plate with microplate sealing film and incubate at 37℃ for 1 hour.

**8. Washing**

Remove the remaining solution by aspiration, add 300 μL of **Wash buffer** to each well, gently tap the plate for 1 minute, remove any remaining **Wash Buffer** by aspirating or decanting, invert the plate and blot it against paper towels. **Repeat the wash step above for three times**.

**9. Streptavidin-HRP Labeling**

1) Dilute **Streptavidin-HRP** stock solution (50 μg/mL) to 0.1 μg/mL with **Dilution Buffer** to make **Streptavidin-HRP** working solution.
2) For all wells, add 100 μL **Streptavidin-HRP** working solution, seal the plate with microplate sealing film and



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incubate at 37℃ for 1 hour, **avoid light**.

**10. Washing**

Repeat step 8.

**11. TMB Substrate Reaction**

Add 200 µL **TMB Substrate Working Solution** to each well. Seal the plate with microplate sealing film and incubate at 37℃ for 20 minutes, **avoid light**.

**12. Termination**

Add 50 µL **Stop Solution** to each well, and tap the plate gently for 3 minutes to allow thorough mixing.

***Note:*** *the color in the wells should change from blue to yellow.*

**13. Data Recording**

Read the absorbance at 450 nm using UV/Vis microplate spectrophotometer.

***Note****: the plate may be read at 600 nm without adding 1 M sulfuric acid, but the Signal-to-Background ratio may be reduced.*



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**FIGURE 1. PREPARATION OF 1:2 SERIAL DILUTIONS OF THE Anti-SARS-COV-2 NEUTRALIZING ANTIBODY**

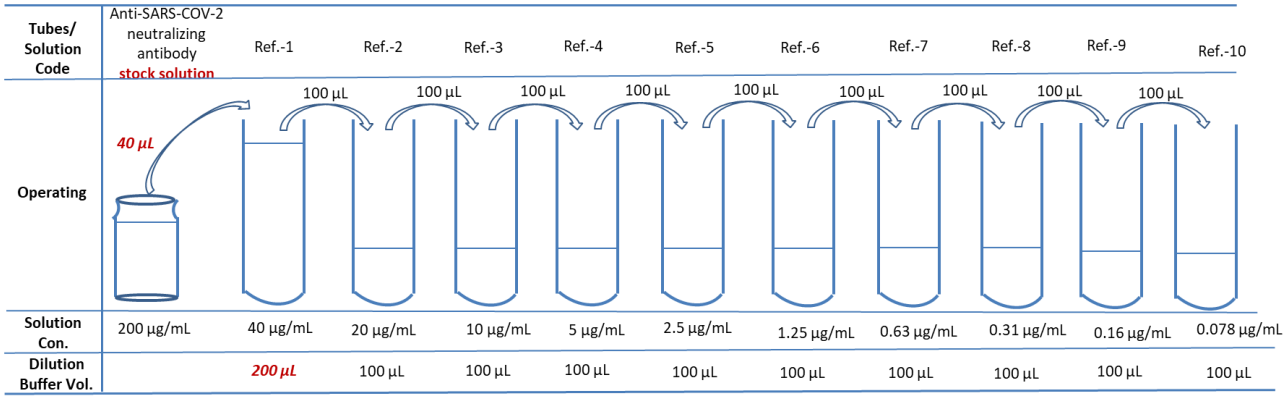
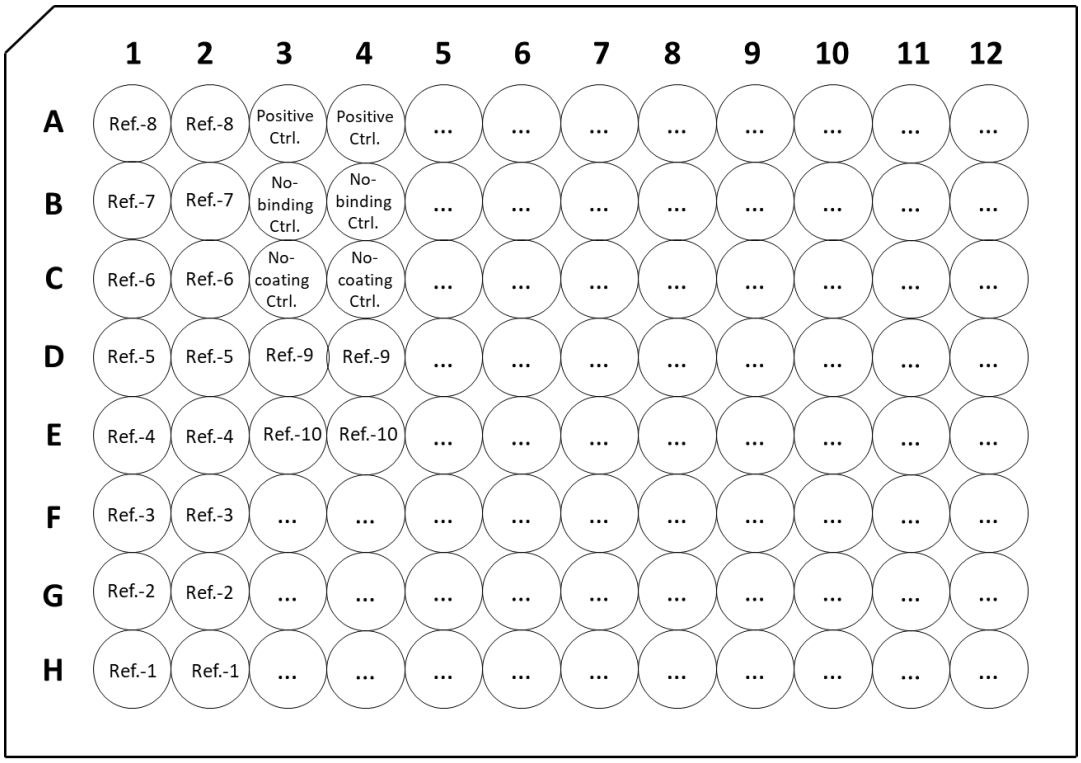



**FIGURE 2. PLATE LAYOUT**






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**TABLE 3. ASSAY PROTOCOL**

| Steps Code | Steps | Reagents & Instruments | Reaction Conditions | Samples | No-binding Control | No-coating Control | Positive Control |
|---|---|---|---|---|---|---|---|
| 1 | Preparation | N/A | N/A | N/A | N/A | N/A | N/A |
| 2 | Coating | SARS-CoV-2 S protein RBD Working Solution | 4℃ for overnight | 100 µL | 100 µL | — | 100 µL |
| 3 | Washing | Wash Buffer 1 | Wash for 3 times | 300 µL | 300 µL | 300 µL | 300 µL |
| 4 | Blocking | Blocking Buffer | 37℃ for 1.5 hours | 300 µL | 300 µL | 300 µL | 300 µL |
| 5 | Washing | Wash Buffer 1 | Wash for 3 times | 300 µL | 300 µL | 300 µL | 300 µL |
| 6 | Binding | Biotinylated Human ACE2 Working Solution | Incubate at 37℃ for 1 hour | 50 µL | — | 50 µL | 50 µL |
| | | Dilution Buffer | | — | 50 µL | — | — |
| 6 | Add Samples | Samples | | 50 µL | 50 µL | | 50 µL |
| | | Dilution Buffer | | — | — | 50 µL | |
| 8 | Washing | Wash Buffer | Wash for 3 times | 300 µL | 300 µL | 300 µL | 300 µL |
| 9 | Streptavidin-HRP Labeling | Streptavidin-HRP Working Solution | 37℃ for 1 hours | 100 µL | 100 µL | 100 µL | 100 µL |
| 10 | Washing | Wash Buffer | Wash for 3 times | 300 µL | 300 µL | 300 µL | 300 µL |
| 11 | TMB Substrate Reaction | TMB Substrate Working Solution | 37℃ for 20 minutes | 200 µL | 200 µL | 200 µL | 200 µL |
| 12 | Stop the Reaction | Stop Solution | Mix by gentle tapping for 3 minutes | 50 µL | 50 µL | 50 µL | 50 µL |
| 13 | Data Recording | UV/Vis spectrophotometer | Measure absorbance at 450 nm, with the correction wavelength set at 600 nm | | | | |

***Note for TABLE 3:***

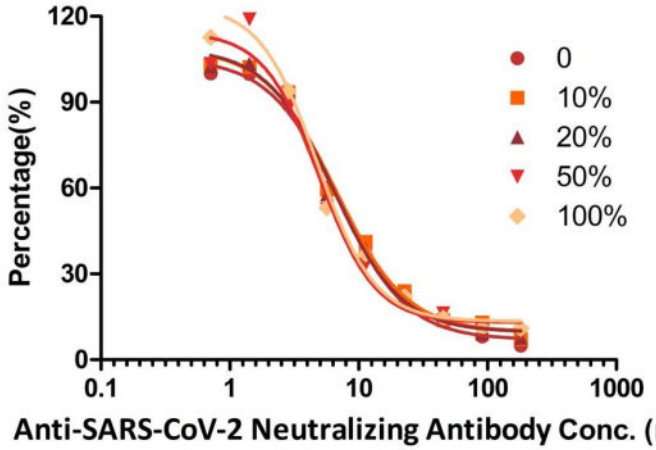
1) ***Samples:*** *Your samples of interest.*
2) ***No-Binding Control:*** *Reaction without* ***Biotinylated Human ACE2*** *added. The absorbance should be around 0.05(< 0.1) at 450 nm.*
3) ***No-Coating Control:*** *Reaction without* ***SARS-CoV-2 S protein RBD*** *coated on the wells. The absorbance should be around 0.05(< 0.1) at 450 nm.*
4) ***Positive Control:*** *Determined the max value in 450 nm absorbance, when out of inhibitors.*
5) *It is recommended that all samples, controls and references should be done in duplicates.*



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## METHOD VERIFICATION

**Determination of Anti-SARS-CoV-2 Neutralizing Antibody titer in different concentrations of human serum.**




Neutralizing titer of Anti-SARS-CoV-2 Neutralizing Antibody, Human IgG1 (Cat. No. SAD-S35) measured by Anti-SARS-CoV-2 neutralizing antibody titer serologic assay kit (Cat. No. TAS-K003) in different concentrations of human serum (CV < 30%).



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**TROUBLESHOOTING GUIDE**

| Problem | Possible Cause | Solutions |
|---|---|---|
| Signal of positive control is weak or abnormal | **Reconstituted protein be aliquoted to less than 5 μg per vial** | ✧ **Reconstituted protein must NOT be aliquoted to less than 5 μg per vial.** |
| | **The working solution not be prepared immediately before use** | ✧ **The working solution should be prepared immediately before use and should not be stored.** |
| | **BiotinylatedACE2, 2019-nCoV S protein RBD** or **Streptavidin - HRP** may have lost activity | ✧ Make sure your proteins are aliquoted into single-use aliquots.<br>✧ Increase the time of reaction or increase the protein concentration may help in case the protein activity is decreased over time. |
| | Errors in instrument settings | ✧ Please check instrument setting. |
| | **Substrate Stock Solution** is outdated; Incubation temperature is incorrect; Incubation time is not sufficient; Repeated freeze-thaw cycles; | ✧ Make sure the **Substrate Stock Solution** is working.<br>✧ Use proper incubation time and temperature. |
| | Pipetting errors | ✧ Make sure that the pipette is calibrated and working properly. |
| High background | Insufficient washing or blocking | ✧ Be sure the blocking step is performed.<br>✧ Increase number of washes and the volume **Wash Buffer** used.<br>✧ Increase Tween-20 concentration to 0.1% in **Wash Buffer**.<br>✧ Make sure **Streptavidin-HRP** is diluted in **Blocking Buffer**. |
| | Sample solvent contains inhibiting factors | ✧ Run a negative control assay with the solvent alone.<br>✧ Maintain DMSO level at <1%. Increase protein incubation time. |
| | Contamination | ✧ Make sure buffers and samples are prepared, used and stored correctly. |
| | The **TMB Substrate Working Solution** is not fresh | ✧ **TMB Substrate Working Solution** must be used within 15 minutes after preparation. |
| Colorimetric signal is erratic | Inconsistent pipetting or dilution methods | ✧ Make sure pipettors are functioning properly and use a multichannel pipettor if possible.<br>✧ Use master mixes to minimize errors.<br>✧ Run duplicates for all tests. |
| | **TMB Substrate Working Solution** is not completely mixed with the reaction solution | ✧ Make sure that **TMB Substrate Working Solution** is adequately mixed with the reaction solution. |
| | Bubbles in the wells | ✧ Tap plate gently to disperse bubbles. |
| | Signal is too high | ✧ The concentration of the samples should be adjusted to achieve optimal reading.<br>✧ Decrease colorimetric HRP substrate incubation time. |
| Inadequate color development | Incomplete removal of residual buffers during previous steps | ✧ Wells should appear dry after aspiration. |
| | Problems with conjugate or color reagents | ✧ Color should appear immediately after the reagent is added. Make sure no contamination or residual buffers in the wells before you start the color development process. |



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