SARS-CoV-2 Spike S1 protein-Coated Plates ELISA Kit

Cat#: orb668904 (ELISA Manual)

Specifications

Table 1. plate details

Items	Specifications
Material	Polystyrene
Color	Clear
Plate Blocking:	2% BSA Blocking Buffer
Formulations	Clear, 96-well plates, coated with 100µL of Streptavidin tetramer, blocked with 300µL of 2% BSA Blocking Buffer and captured 0.1 µg/well of biotinylated SARS-CoV2 Spike S1 protein.
Detection Method	Colorimetric
Type	Detection Plate, Biopanning, ELISA

Assay Principles

Streptavidin (SA) has an extraordinarily high affinity for biotin with a dissociation constant (Kd) on the order of 10−14mol/L, the Biotinylated molecules can bind to the SA irreversibly. The SARS-CoV2 Spike S1 protein-Coated Plates are immobilized with biotinylated SARS-CoV2 Spike S1 protein to a Streptavidin tetramer protein coated plate, which is easy to use and widely available for applications.

Example ELISA Procedure

Materials and Reagents Preparation

Before starting the ELISA Assay, we should prepare the all reagents and materials required in the experiment. You can prepare these regents by following operations, we also provide the matching reagent kit

Wash Buffer: PBS or TBS with 0.05% (v/v) Tween-20 (usually at pH7.4), 500 mL is sufficient for 96 tests.
Dilution Buffer: Wash Buffer with 0.5% (w/v) bovine serum albumin (BSA) (i.e. Jackson, Catalog#. 001-000-162), 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: 20 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 125 µL substrate stock solution in 25 mL substrate dilution buffer and add 20 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.
Microplate sealing film (Sigma-Aldrich, Catalog # Z724742)

Pipettes and pipette tips
UV/Vis microplate spectrophotometer (absorbance 450 nm, correction wavelength set to 630 nm).

Recommended Protocol
1.Preparation
Reconstitute and store all reagents as recommended.
Open the plate package and take out the corresponding quantity of detachable 8-well stripsaccording to your experimental design.

2.Add antibody or ACE2 protein samples
1)Make series dilution of the ACE2 protein or antibodies as appropriate with **Dilution Buffer.**
2)Add 100 µL of the serial dilution of sample to each well, incubate at 37°Cor RT for 1 hour.
3)For the" Blank" wells, please add 100 µL **Dilution Buffer**.

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.Add primary antibody
1)Dilute **primary antibody** to an appropriate concentration with Dilution Buffer.
2)For all wells, add 100 µL of diluted **primary antibody**, and incubate at 37°Cor RT for 1 hour.
If you don't need a primary antibody and only need a secondary antibody, you can add enzyme-labeled secondary antibody refer to step 6.

5.Washing
Repeat step 3.

6.Add enzyme-labeled secondary antibody
1)Dilute secondary antibody to an appropriate concentration with Dilution Buffer.
2)For all wells, add 100 µL of diluted **secondary antibody**, and incubate at 37°Cor RT for 1hour, **avoid light**.

7.Washing
Repeat step 4.

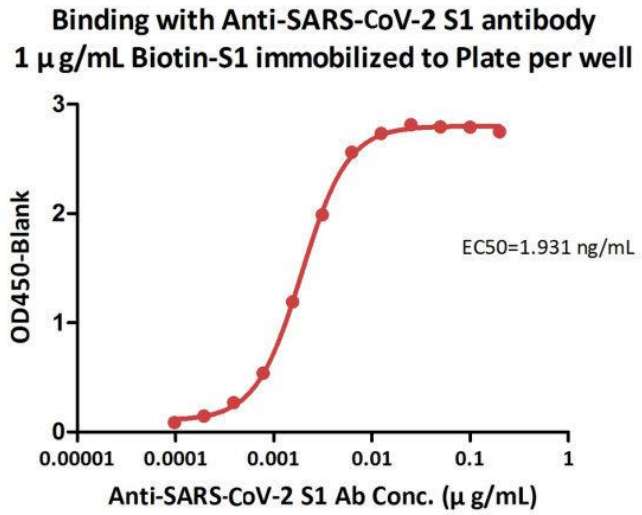
8.TMB Substrate Reaction
Add 200 µL **TMB Substrate Working Solution** to each well. Seal the plate with microplate sealing film and incubate at 37°Cor RT for 20 minutes, **avoid light**.

9.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.*

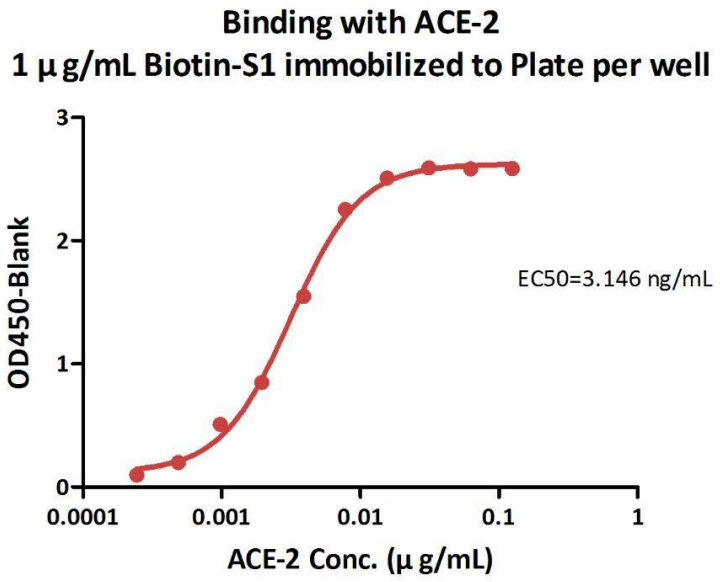
10.Data Recording
Read the absorbance at 450 nm using UV/Vis microplate spectrophotometer.
Note*: the plate may be read at 600 nm without adding 1 M sulfuric acid, but the Signal-to-Background ratio may be reduced.*

Example Data of ELISA Binding Assay

1. Binding Assay between S1 protein and anti-SARS-CoV-2 S1 antibody Immobilized biotinylated SARS-CoV2 Spike S1 protein at 1 µg/mL (100 µL/well) on Streptavidin Coated Plates, Clear, 96-Well, can bind anti-SARS-CoV-2 S1 antibody with a linear range of 0.1-3 ng/mL (QC tested).



2. Binding Assay between S1 protein and ACE-2 protein
Immobilized biotinylated SARS-CoV2 Spike S1 protein at 1 µg/mL (100 µL/well) on Streptavidin Coated Plates, Clear, 96-Well (Cat. No. SP-11), can bind ACE2 with a linear range of 0.2-4 ng/mL (QC tested).



Example Biopanning Procedure
Materials and Reagents Preparation
Before starting the Biopanning, you should prepare the all reagents and materials required in the experiment, including your clones.

Wash Buffer: PBS with 0.05% (v/v) Tween-20 (usually at pH7.3~7.4), 500 mL is sufficient for 96 tests.
Elution Buffer: 10 mM Glycine-HCl, pH 2.2~2.5.
Neutralization Buffer: 1 M Tris-HCl, pH 9.1.

Recommended Protocol
1. Preparation
Reconstitute and store all reagents as recommended.
Open the plate package and take out the corresponding quantity of detachable 8-well strips according to your experimental design.

2. Washing
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 six times**.

3. Add your clones to wells
The anti-SARS-coV-2 Phage Clones were sequentially added to the plate wells, and shaken gently at room temperature for 2 h.

4. Washing
Wash the plate with **Wash buffer for 10 times refer to step 2.**

5. Elute the target clones from the plate
Elute the plate-bound phage clones with **Elution Buffer** (10 mM Glycine-HCl, pH 2.2~2.5).

6. Neutralization
Neutralized the clones in plate wells with **Neutralization Buffer** (1 M Tris-HCl, pH 9.1).
After determining the titer, the eluate was amplified and purified for the next round of screening.
Two additional rounds of selection were performed under more stringent conditions, in which plates were washed with a higher concentration of PBST (0.1% and 0.3% for second and third round, respectively) for a longer period (10*2 min and 10*3 min for second and third round, respectively). After the third round of selection, the phage clones were subjected to ELISA analysis.

7. ELISA Assay for Selecting Positive Phage Clones
The ELISA Assay refer to "Example ELISA Procedure".
After wash the plate 3 times with Wash Buffer (PBS, with 0.05% Tween-20), phage clones (i.e. 1010 pfu/well) and control phage were added to plate wells and incubated at room temperature for 1 h. After washing 3 times with Wash Buffer, 200 mL of horseradish peroxidase (HRP)-anti-Human IgG (1:10000) was added and the plates were incubated for another hour at room temperature. The plates were washed again with Wash Buffer, and add 200 µL TMB Substrate Working Solution to each well, seal the plate with microplate sealing film and incubate at room temperature for 20 minutes, avoid light. The reaction was terminated later by adding 50 µL/well of Stop Solution (1 mol/L H2SO4), and the absorbance was measured at 450 and 655 nm.

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solutions
Signal of positive control is weak or abnormal	**Incorrect storage of plate**	**The plate should be store plates at 4°C, once you open the package, get the amount you need and keep the rest airtight.**
Detection Antibody is outdated or no prepared the working solution immediately before use	**The working solution should be prepared immediately before use and should not be stored.**	
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. Use proper incubation time and temperature.	
Pipetting errors	Make sure that the pipette is calibrated and working properly.	
High background	Serum samples	If you want test serum samples, the BSA Blocking plate is not suitable for this purpose. **You can use specific Blocking Buffer blocking plate to avoid the background**

Sample solvent contains inhibiting factors	Run a negative control assay with the solvent alone. 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. Use master mixes to minimize errors. 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. 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.	