



Anti-SARS-CoV-2 Spike glycoprotein Antibody (CSB-RA33245A1GMY) Neutralising ELISA Protocol

1. Coating

Coat the plate with 0.2µg/well (2µg/ml, 100µl/well) SARS-CoV-2 Spike glycoprotein (CSB-YP3324GMY1) at 4°C overnight. The protein is diluted in pH9.5 coating buffer, namely Carbonic acid buffer (0.035 mol/L NaHCO₃, 0.015mol/L Na₂CO₃).

2. Washing

Set the enzyme labeling board at room temperature for 10min. Remove the protein solution by decanting or aspirating. Make sure that the solution in wells is completely removed. Then wash the wells with 200µl/well PBS (pH7.4) for 1 time and aspirate the solution, invert the plate and sit it on multilayer clean paper towels for a while to remove the solution completely.

3. Blocking

Block the wells with 200µl/well blocking buffer (4% Non-fat milk powder in PBS) at 37°C for 1h. After blocking, aspirate the solution in the wells, invert the plate and sit it on multilayer clean paper towels for a while to remove the solution completely.

4. Adding antibody

Add 100µl/well 240000 – 0.915527 ng/ml (gradient dilution, 19x) Anti-SARS-CoV-2 Spike glycoprotein Antibody (CSB-RA33245A1GMY) and 1000ng/ml (gradient dilution, 19x) ACE2 protein-HRP conjugate (CSB-MP866317HU) to each well, and seal the plate with sealing film. Then put the plate at 37°C and incubate for 1h.

5. Washing

Wash the wells with 200µl/well PBST (0.1% Tween-20 in PBST, pH7.4) for three times firstly, and then wash the wells with 200µl/well PBS for three times. Then aspirate the solution, invert the plate and sit it on multilayer clean paper towels for a while to remove the solution completely.

6. Adding substrate

Add 100µl TMB solution to each well and incubate at 37°C for 10-20min.

7. Termination

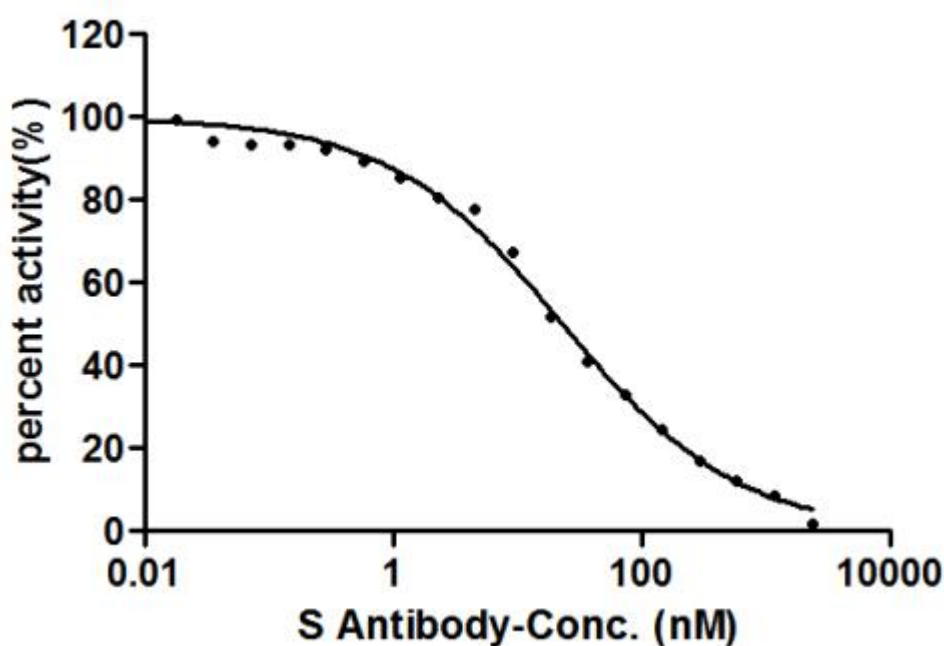
Add 50µl/well 2M H₂SO₄ solution to the plate to terminate the reaction.

8. Read OD

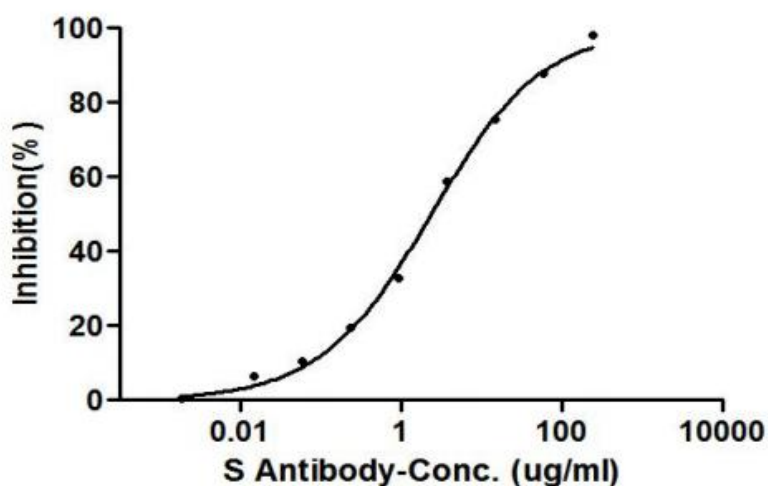
Immediately put in the enzyme label and read at 450 nm.

Reference Data

Coating	SARS-CoV-2 Spike glycoprotein (CSB-YP3324GMY1)	0.2µg/well
Reaction Antibody 1	ACE2 protein-HRP conjugate (CSB-MP866317HU)	0.1µg/well
Reaction Antibody 2	Anti-SARS-CoV-2 Spike glycoprotein Antibody, HRP conjugated (CSB-RA33245A1GMY)	240000 – 0.915527ng/ml 100µl/well
Result	IC ₅₀	20.49 to 26.56(23.32 nM)
Result	IC ₅₀	1.963 to 2.885(2.38µg/ml)



S Antibody	
µg/ml	Inhibition(%)
240	98.22%
60	87.70%
15	75.51%
3.75	58.82%
0.9375	32.69%
0.234375	19.35%
0.05859375	10.43%
0.014648438	6.58%
0.001831055	0.53%



IC₅₀=2.38 ug/ml