



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

1. Coating

Coat the plate with 0.2 μ g/well (2 μ g/ml, 100 μ l/well) SARS-CoV-2 Spike glycoprotein his and flag tag (CSB-MP3324GMY) at 4 $^{\circ}$ 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 400 μ l/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 400 μ l/well blocking buffer (4% Non-fat milk powder in PBS) at 37 $^{\circ}$ C for 2h. 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 100 μ l/well 0.7629 - 100000ng/ml (gradient dilution, 2x to 18x) Anti-SARS-CoV-2 Spike glycoprotein Antibody (CSB-RA33245A1GMY) to each well, and seal the plate with sealing film. Then put the plate at 37 $^{\circ}$ C and incubate for 1h.

5. Washing

Wash the wells with 400 μ l/well PBST (0.1% Tween-20 in PBST, pH7.4) for three times firstly, and then wash the wells with 400 μ l/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 detection antibody

Dilute the Peroxidase-Affinipure Goat Anti-Human IgG, Fc γ Fragment Specific (CSB-PA160506) by 4% non-fat milk powder in PBS at 1:10000. Add 100 μ l/well on the plate and incubate at 37 $^{\circ}$ C for 1h.

7. Washing

Repeat step 5

8. Adding substrate

Add 100 μ l TMB solution to each well and incubate at 37 $^{\circ}$ C for 10-20min.

9. Termination



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

10. Read OD

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

Reference Data

Coating	SARS-CoV-2 Spike glycoprotein his and flag tag (CSB-MP3324GMY)	0.2µg/well
Reaction Antibody	Anti-SARS-CoV-2 Spike glycoprotein Antibody (CSB-RA33245A1GMY)	0.7629 - 100000ng/ml 100µl/well
Detection	Peroxidase-Affinipure Goat Anti-Human IgG, Fcγ Fragment Specific (CSB-PA160506)	1:10000
Result	EC ₅₀	36.79 to 48.87ng/ml

