



## Anti-SARS-CoV-2 Spike glycoprotein Antibody, HRP conjugated(CSB-RA33245B1GMY) ELISA Protocol

### 1. Coating

Coat the plate with 0.2μg/well (2μg/ml, 100μl/well) SARS-CoV-2 Spike glycoprotein (CSB-MP3324GMY1) at 4°C overnight. The protein is diluted in pH9.5 coating buffer, namely Carbonic acid buffer (0.035 mol/L NaHCO<sub>3</sub>, 0.015mol/L Na<sub>2</sub>CO<sub>3</sub>).

### 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 0.7629 – 100000ng/ml (gradient dilution, 2x to 18x) Anti-SARS-CoV-2 Spike glycoprotein Antibody, HRP conjugated (CSB-RA33245B1GMY) 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 H<sub>2</sub>SO<sub>4</sub> 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-MP3324GMY1)	0.2µg/well
Reaction Antibody	Anti-SARS-CoV-2 Spike glycoprotein Antibody, HRP conjugated (CSB-RA33245B1GMY)	0.7629 – 100000ng/ml 100µl/well
Result	EC <sub>50</sub>	298.5 to 368.1ng/ml

