Spike & ACE2-blocking activities assay

1. Recombinant human ACE2 was diluted to a concentration of 5μg/ml with 0.05 M PH 9.6 carbonate buffer [1]. Then add 100 μL to each well of polystyrene plate at 4°C overnight.

2. Aspirate the solution in the well and wash each well three times with Wash Buffer [2]. Complete removal of liquid at each step is essential to good performance. Remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

3. Add 1% polyvinyl alcohol (PVA) and block at room temperature for 1 hour.

4. Repeat the aspiration/wash as in Step 2.

5. Add a pre-incubated mixture of the anti-spike antibody titrated into a constant amount of spike (1μg/ml) p er well for 2-hour competitive binding at room temperature.

6. Repeat the aspiration/wash as in Step 2.

7. Add 100 μL of Detection Antibody in working concentration to each well.
Cover/seal the plate and incubate for 1 hour at room temperature or 37℃ for 0.5 ~ 1 hour.

8. Repeat the aspiration/wash as in Step 2.

9. Add 200 μL of temporarily prepared TMB Substrate Solution to each well.
Incubate for 20 minutes at room temperature. Protect from light.

Tel: +86-21-50478399
Email: support@genemedi.net

10. Add 50 μL of 2M sulfuric acid Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

11. Determine the optical density of each well within 20 minutes, using a microplate reader set to 450 nm. The O·D value of each well is measured after the blank control well is zeroed.

[1] PH9.6 0.05M carbonate buffer:

Na2CO3	1.59 g
NaHCO3	2.93 g

Add distilled water to 1000ml

[2] Wash buffer (PH7.4 PBS):

KH2PO4	0.2 g
Na2HPO4·12H2O	2.9 g
NaCl	8.0 g
KCl	0.2 g
Tween-20	0.5ml

Add distilled water to 1000ml