

## **Protocol of SARS-CoV2 (2019nCoV) NP paired antibodies sandwich ELISA**

1. The Capture Antibody #1 was diluted to a concentration of 1 ~ 10 ug/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] (300 µL/well). 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 5% non-fat milk and block at 37 °C for 1 h.
4. Repeat the aspiration/wash as in Step 2.
5. Add 100 µL of each serially diluted protein standard or test sample per well including blank, negative control and positive control. Ensure reagent addition is uninterrupted and completed within 15 minutes. Cover/seal the plate and incubate for 2 hours at room temperature or 37 °C for 1 hour.
6. Repeat the aspiration/wash as in Step 2.
7. Add 100 µL of Detection Antibody #2 in working concentration to each well. Cover/seal the plate and incubate for 1 hour at room temperature or 37 °C 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.

10. Add 50  $\mu$ L 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 Capture Antibody: Nab001 or Nab002*

*#2 Detection Antibody: Nab002 (if Nab001 is used as Capture Antibody) or Nab001 (if Nab002 is used as Capture Antibody)*

**[1] PH9.6 0.05M carbonate buffer:**

Na<sub>2</sub>CO<sub>3</sub> 1.59 g

NaHCO<sub>3</sub> 2.93 g

Add distilled water to 1000ml

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

KH<sub>2</sub>PO<sub>4</sub> 0.2 g

Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O 2.9 g

NaCl 8.0 g

KCl 0.2 g

Tween-20 0.5ml

Add distilled water to 1000ml