TC4-SOP 0-2b ELISA titer test

Project Name: _____

MATERIALS:

- **Blocking Buffer**
  Gelatin-NET
  10% FBS in 1X PBS
- **Coating Buffer - 0.1 M Sodim Carbonate, pH 9.5**
  7.13g $NaHCO_3$, 1.59g Na_2CO_3; q.s. to 1.0 L; pH to 9.5 with 10 N NaOH. Freshly prepare or use within 7 days of preparation, stored at 2-8℃
- **PBST buffer**
  PBS buffer with 0.05%(v/v) Tween-20
- **Test antibody samples (1st antibody)**
- **2nd antibody (HRP-goat-anti-mouse)**
- **TMB substrate - Tetramethylbenzidine (TMB) and Hydrogen Peroxide**
  KPL SureBlue™ TMB Microwell Peroxidase Substrate (Cat. No. 52-00-00).
  BD Pharmingen™ TMB Substrate Reagent Set (Cat. No. 555214).
- **Stop Solution**
  TMB – 1 M H_3PO_4 or 2 N H_2SO_4.
- **ELISA plate (NUNC 442404)**
- **12-channel pipette**
- **ELISA reader**

METHODS:

☐ **Coat plate with antigen**：

1. Prepare an antigen solution in Coating Buffer at 0.2~10 μg/ml. The concentration of antigen is usually 10 μg/ml. Prepare ~10 ml antigen solution for each plate.
2. Using a 12-channel pipette and tips, dispense 100 μL antigen solution into each well of ELISA plate.
3. Seal coated plates using 96-well adhesive plastic seal and incubate overnight at room temperature (RT) or 2 hr at 37℃.

- Prepare control: **NC**-Coating buffer without antigen；**PC**-Coating buffer with sera.

☐ **Block**：Aspirate wells and wash 3 times PBST buffer. After last wash, invert plate and blot on absorbent paper to remove any residual buffer. Block plates with ≥ 200 μL/well blocking buffer. Incubate at RT for 1 hour. Aspirate and wash 3 times with PBST.

☐ **Add 1st Antibody:** Pipette 100 μL of each sample and control diluted in blocking buffer into appropriate coated wells. Seal plate and incubate ≥ 1 hr at RT. Aspirate and wash 3 times with PBST.

☐ **Add 2nd Antibody**：Pipette 100 μL of 5000X 2nd Antibody diluted in blocking buffer to each well. Seal plate and incubate for 1 hr at RT. Aspirate and wash 7 times with PBST buffer.

☐ **Add TMB substrate**：Add 100 μL of TMB substrate solution to each well. Incubate plate (without plate sealer) for 10~30 min at RT in the dark and then add 50 μL of Stop solution to each well.

☐ **Optical density**：Using ELISA reader to read absorbance at 450 nm within 30 min of stopping reaction. If wavelength correction is available, subtract absorbance 570 nm from absorbance 450 nm.

Note:

_____

_____

_____

Date	Operator	QC