ELISA Protocol

Purpose:

Detect and quantify immune reactions by binding soluble antigens or antibodies to a solid-phase carrier, such as polystyrene. This protocol evaluates binding affinity for antigen/antibody or ligand/receptor interactions.

Materials and Reagents:

- **Antigen Protein:** Full length protein-synthetic nanodisc
- **Primary antibody:** Anti-Flag monoclonal antibody
- **HRP-conjugated secondary antibody**
- **Coating buffer solution (CBS):** 15 mmol/L Na_2CO_3, 35 mmol/L $NaHCO_3$, pH9.6
- **Washing buffer** (PBST): 1×PBS with 0.1% Tween 20
- **Blocking solution**(2% BSA): 2g BSA in 100ml PBST, thoroughly mixed
- **Dilution solution**(1% BSA): 1g BSA in 100ml PBST, thoroughly mixed
- **Substrate Solution:** 8 µl 3% H_2O_2 and 100 µl 10 mg/mL TMB in 10 mL Substrate Solution A (50 mmol/L $Na_2HPO_4 \cdot 12H_2O$, 25 mmol/L Citric acid, pH5.5).
- **Stop Solution:** 1 mol/L sulfuric acid

Experimental Steps:

1. Coat the plate with 0.2 µg/well (2 µg/ml, 100 µl/well) antigen protein at 4 ℃ for overnight (or 16 hours) in Coating Buffer (15 mmol/L Na_2CO_3, 35 mmol/L $NaHCO_3$, pH9.6).
2. Blocking: Remove the coating solution, tap the plate gently, and block with blocking solution (2% BSA) at 200µl per well. Incubate at 37℃ for 1 hour.
3. Primary Antibody Incubation: Discard the blocking solution, tap the plate gently, dilute the anti-Flag primary antibody in dilution solution, and add 100µl per well. Incubate at 37℃ for 1 hour.
4. Washing: Discard the primary antibody, Wash the wells with 300 µl per well washing Buffer for 4 times. Ensure the complete removal of the washing buffer.
5. Secondary Antibody Incubation: Dilute the HRP-conjugated secondary antibody in dilution solution, add 100µl per well, and incubate at 37℃ for 1 hour.
6. Washing: Discard the secondary antibody, wash three times with PBST, and tap the plate gently.
7. Color Development: Add 100 µl Substrate Solution into each well, incubate

at 37 ℃ for 10 min. Avoid light.

8. Termination and Detection: Add 100µl of the stop solution per well to terminate the reaction and measure using the ELISA reader (OD450).