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:** Human STEAP2 full length protein-synthetic nanodisc (Catalog No. FLP100043) solution
- **Primary antibody:** Anti-Flag monoclonal antibody
- **HRP-conjugated secondary antibody**
- **Coating buffer solution (CBS):** 15mmol/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:** 8μl 3% H_2O_2 and 100μl 10 mg/mL TMB in 10mL 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 (FLP100043) at 4℃ for overnight (or 16 hours) in Coating Buffer Solution (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

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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 100μl 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).

Example:

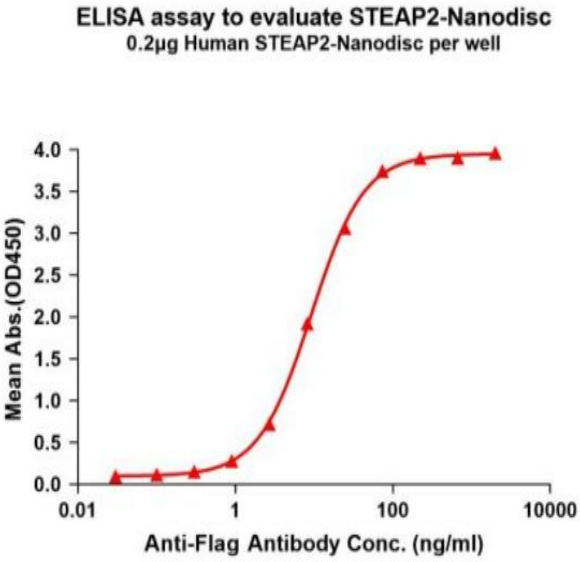

Figure1. Elisa plates were pre-coated with Flag Tag STEAP2-Nanodisc (0.2μg/per well). Serial diluted anti-Flag monoclonal antibody solutions were added, washed, and incubated with secondary antibody before Elisa reading. From above data, the EC50 for anti-Flag monoclonal antibody binding with STEAP2-Nanodisc is 9.198ng/ml