SOP: SP039

ELISA Assay

Materials and Reagents:

1. 96 well ELISA plate
2. Multi-channel pipettor
3. 1-200µl pipet tips
4. TBST (note 1)
5. TBS (note 2)
6. Blocking solution (note 3)
7. KPL *p*-NPP developer kit (cat# 508000)
8. Primary antibody being tested
9. Antigen testing against
10. Secondary antibody
11. 4°C cold room or fridge
12. Plate sealer

Protocol:

1. _____ Coat 96 well ELISA plate with appropriate antigen or sample (note 4).

2. _____ Incubate ELISA plate overnight at 4°C.

3. _____ Remove antigen and save for another assay if needed.

4. _____ Block ELISA plate with 200µl of blocking solution for 1 hour.

5. _____ Dump off blocking solution into sink.

6. _____ Transfer primary antibody to the ELISA plate, 100µl/ well (note 5).

7. _____ Incubate for 1½ -2 hours at room temperature.

8. _____ Discard the samples into sink or save primary antibody if needed.

9. _____ Wash the plates with 100-200µl of TBST five times and on the fifth wash let stand for ten minutes.

10. _____ Prepare secondary antibody: use anti-mouse alkaline phosphatase conjugated antibody at 1:2500 in TBS (note 6).

11. _____ Plate 100ul of the secondary antibody and incubate for 1½ -2 hours.

12. _____ Discard secondary in sink.

13. _____ Wash the plate with TBS fives times and on the fifth wash let stand for ten minutes.

14. _____ Prepare 10 ml of KPL *p*NPP developer per 96 well plate: 2 ml of Diethanolamine Buffer in 8 ml of ddH20 and add 1 *p*NPP tablet.

15. _____ Add 100µl of developer to each well and incubate at 37°C until reaction occurs (note 7).

16. _____ Read at 405nm on a microplate reader.

17. _____ Allow the developer to dry in a chemical hood before discarding the ELISA plate.

Notes:

1. TBST is prepared with 1.21g Tris, 8.77 g NaCl, pH 7.4, 2.5 ml 20% Tween 80 or 0.5 ml Tween 80, QS to 1L with ddH2O.
2. TBS is prepared with 1.21g Tris, 8.77 g NaCl, pH 7.4, QS to 1L with ddH2O.
3. 1-2% BSA in TBST.
4. Prepare a stock solution of antigen by add 100μg of protein to 10 ml of PBS. Mix well and coat 100μl of antigen per well, if using pure protein. The concentration of antigen can increase or decrease depending on individual assays. This antigen can be reused several times. Store at -20°C between each use. Be sure to include a positive and negative control.
5. If needed dilute the primary antibody to proper titer. Primary antibodies can be used more than once. Store at -20°C between each use.
6. If a mouse monoclonal antibody is not used for primary be sure to use the appropriate secondary. The secondary must be alkaline phosphatase conjugated to use this developing kit.
7. The development usually takes between 10-30 minutes.