SOP: SP040.1

Capture ELISA Assay with HRP Substrate

Materials and Reagents:

1. 96 well ELISA plate
2. Multi-channel pipettor
3. 1-200µl pipet tips
4. TBST (note 1)
5. 1% BSA-TBST
6. 0.1% BSA-TBST
7. Biotinylated detection antibody
8. Streptavidin-HRP antibody (Zymed)
9. Capture antibody
10. TMB + Substrate - Chromogen developer (Dako #S1599)
11. Samples to be tested
12. 4°C cold room or fridge
13. Plate sealer
14. 0.18M H_2SO_4
15. Biotek Epoch multiplate reader
16. Plate washer

Protocol:

1. _____Coat 96 well ELISA plate with 100µl capture antibody as per manufacturer's recommended concentration. Be sure to coat enough wells for all samples, and a positive and negative control.

2. _____Incubate ELISA plate overnight at 4°C.

3. _____Use plate washer to aspirate off antibody solution. Wash plate 3x with 300µl/well of TBST.

4. _____Block ELISA plate with 200µl per well of 1% BSA-TBST solution for 1 hour.

5. _____Use plate washer to aspirate off antibody solution.

6. _____Transfer sample, positive and negative controls to the ELISA plate, 100µl/ well.

7. _____Incubate for 1 ½ -2 hours at room temperature.

8. _____ Use plate washer to aspirate off antibody solution..

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

10. _____Prepare detection biotinylated antibody as per manufacture's recommended concentration in 0.1% BSA in TBST.

11. _____Plate 100µl of the detection antibody and incubate for 1½ -2 hours.

12. _____Discard secondary in sink.

13. _____Wash the plate with TBST fives times and on the fifth wash let stand for ten minutes.

14. _____Prepare the streptavidin-HRP conjugated antibody at a dilution of 1:2500 in 0.1% BSA in TBST, add 100 µl per well.

15. _____ Incubate at room temperature for 1 hour.

16. _____ Bring 10ml of TMB substrate to room temperature per ELISA plate.

17. _____ Add 100µl/well of developer and watch for color change ~15-30minutes.

18. _____ After development, stop the reaction with 100µl/well of 0.18M H_2SO_4.

19. _____ Read at 450nm on a microplate reader.

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

Notes:

1. <u>TBST</u>
   1.21g Tris
   8.77 g NaCl
   pH 7.4
   2.5 ml 20% Tween 80 or 0.5 ml Tween 80
   q.s. to 1L with MilliQ H_2O.

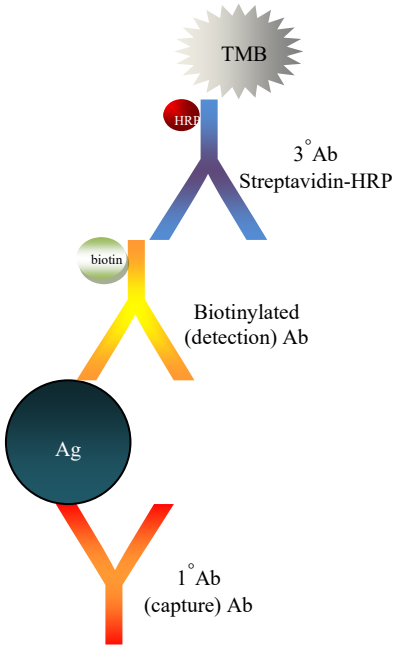

Figure 1 – Diagram of Indirect (Sandwich) ELISA with HRP Substrate