

SOP: SP040

Capture ELISA Assay

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

Protocol:

1. ____ Coat 96 well ELISA plate with 100µl capture antibody as per manufacturer's recommended concentration (note 2).
2. ____ Incubate ELISA plate overnight at 4°C.
3. ____ Discard the capture antibody in the sink.
4. ____ Block ELISA plate with 200µl per well of 1% BSA-TBST solution for 1 hour.
5. ____ Discard the blocking solution into sink.
6. ____ Transfer sample, positive and negative controls to the ELISA plate, 100µl/ well.
7. ____ Incubate for 1 ½ -2 hours at room temperature.
8. ____ Discard the samples into sink.
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 of developer to each well and watch for color change.
18. _____ After development, stop the reaction with 100µl of .18M H₂SO₄.
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. TBST is prepared 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. Be sure to coat enough wells for all samples, and a positive and negative control.