SOP: SP040b

IL-2 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. Detection antibody: Biotinylated rat anti-mouse IL-2 monoclonal (BD PharMingen #18172D)
8. Streptavidin-HRP antibody (Zymed)
9. Capture antibody: purified rat anti-mouse IL-2 monoclonal (BD PharMingen #554424)
10. TMB+ Substrate-Chromogen developer (Dako #S1599)
11. Supernatants from T cell clones
12. IL-2 stock solution
13. 4°C cold room or fridge
14. Plate sealer

Protocol:

1. _____ Coat 96 well ELISA plate with 100µl capture antibody at a concentration of 2µg/ml and cover with a plate sealer (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 blocking solution for 1 hour.

5. _____ Discard blocking solution into sink.

6. _____ Transfer T cell culture supernatants, the IL-2 standards, and the BSA negative control to the ELISA plate, 100µl/ well (note 3).

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

8. _____ Discard the supernatants 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 at a concentration of 1µg/ml 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 antibody at a dilution of 1:2500 in 0.1%BSA-TBST, add 100µl per well.

15. _____ Incubate at room temperature for 1 hour.

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

17. _____ Discard the antibody in the sink.

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

19. _____ Add 100µl of developer to each well and watch for color change.

20. _____ After development, stop the reaction with 100 µl of .18M H_2SO_4.

21. _____ Read at 450nm on a micro-plate reader.

22. _____ 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. Dilute the antibody in .1M sodium phosphate buffer pH 9.0. Make this by adding about 2.2 ml of .1M monobasic sodium phosphate to 500 ml of 0.1M dibasic sodium phosphate buffer. The pH may need to be adjusted a little. Do this by adding more monobasic until pH 9.0 is achieved. Be sure to add 36 wells for a IL-2 standards (positive control) and 1 well for a BSA negative control
3. The stock solution of IL-2 is at 200ng/ml. From this solution, make a standard stock of 20ng/100µl. Take 200ul of this and transfer it to the ELISA well designated for 20ng/100µl. From this, make 2 fold dilutions (diluting in 0.1%BSA-TBST) all the way down to .01ng/µl. It is best to do a set of three standards to make a nice standard curve.