

Enzyme Linked Immunosorbent Assay (ELISA)

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I have used this protocol with following DuoSets from R&D systems: human IL-1ra, human IL-6, human IL-8, human TARC, human PARC, human MCP-4.

Before you start

- Dilute your samples and standarts in the same buffer
- Wash the plates thoroughly and consistently to obtain reliable results
- Always make duplicates of your samples and standards

Solutions needed

- PBS: 137mM NaCl, 2.7nM KCl, 8.1 mM Na₂HPO₄, 1.5 mM KH₂PO₄, pH 7.2-7.4, sterile filtered
- Wash buffer: 0.05% Tween in PBS
- Reagent diluent: 1% BSA in PBS, sterle filtered
- Substrate: ready-to-use TMB substrate from Biomeda
- Stop solution: 0.8 N sulfuric acid

Protocol

1. Dilute coating antibody in PBS 1:180 (for full 96 well plate - mix 58µl antibody and 10.5 ml PBS)
2. Distribute diluted antibody in 96 well plate (MaxiSorp from Nunc) at 100µl/well
3. Incubate at 4°C overnight
4. Aspirate antibody solution from the plate
5. Wash once with 250 µl wash buffer
6. Fill the wells with 250 µl reagent diluent, incubate for 2 h on a shaker, 100rpm at RT
7. Aspirate reagent diluent from the wells, wash once with 250µl wash buffer
8. Add 100 µl samples or standarts per well, incubate 2 h on a shaker at RT
9. Wash the wells 3 times with 250 µl wash buffer
10. Dilute the detection antibody in reagent diluent 1:180 (see above)
11. Add 100 µl of diluted detection antibody to the wells
12. Incubate 2 h on a shaker at RT
13. Wash 4 times with 250 µl wash buffer
14. Dilute streptavidine-HRP conjugate in reagent diluent 1:200 (for 96 well plate - mix 52 µl conjugate and 10.5 ml reagent diluent)
15. Add 100µl of diluted conjugate to each well
16. Incubate 20 min on a shaker at RT
17. Wash 4 times with 250 µl wash buffer
18. Add 50 µl substrate to each well
19. Incubate 10-20 min at RT, control the (blue) color development
20. Add 50 µl stop solution to each well. The color will turn yellow
21. Dtermine the optical density of each well immediately, using a microplate reader at 450 nm, if possible make a wavelength correction at 540 or 570 nm

RT = room temperature