Protocol 501_Mouse Albumin ELISA

Reagents

- ELISA coating buffer: 0.1M NaHCO3 (3.18g Na_2CO_3 + 5.88g $NaHCO_3$ made up to 1000ml with water).
- ELISA blocking buffer (PBS, 2% BSA – NB NO AZIDE – really inhibits HRP!!!!)
- ELISA wash buffer (PBS, 1:2000 Tween-20)
- ELISA substrate buffer: 0.1M NaH2PO4, pH 4 (13.8g NaH_2PO_4 made up to 1000ml)
- Polyclonal Goat-anti-mouse Albumin (Bethyl Labs Cat# A90-134A)
- Polyclonal Goat-anti-mouse Albumin HRP (Bethyl Labs Cat# A90-134P)
- Standard – Mouse reference serum (Bethyl Labs Cat# RS10-101) Contains 45mg/ml albumin. Use top concentration of 1µg per ml i.e. dilute 1:45000! 1:450 per-diluted stock can be made, aliquoted and stored at -20 until needed for 1:100 dilution is this is easier)
- Nunc immunoplates

Method – as for isotype ELISA but:

COAT plates in normal ELISA-coating buffer at pH 9.6 with 1:200 of unlabeled goat-anti-mouse albumin

Block with 2% BSA (no problem with cross-reactivity) in PBS

Add in samples starting at around 25mg/ml of cecal content and dilute 3x down the plates in duplicate

Add standards starting at 1µg/ml (reference serum contains 45mg/ml therefore dilute 1:45000)

Add developing antibody 1:33000 in PBS/BSA

Method – isotype ELISA

1. Coat plates:
    a. Dilute coating antibody 1:1000 in ELISA coating buffer
    b. Plate 50µl per well. Tap the plate to ensure even spreading.
    c. Cover the plates in parafilm and incubate 6hrs (rm temp) to overnight (4ºC) in a humidified box (wet tissue at the bottom!)

2. Block:
    a. Wash plates 3 times by immersion in ELISA wash buffer
    b. Add 150µl of 2% BSA/PBS per well. Incubate for 15mins to several hours (plates can be frozen like this and stored long-term)

3. Add samples:
    a. Plan out how to plate your samples and standards. (page 3!)
    b. Make standard dilutions of standards and samples in blocking buffer, leaving a final volume in the wells of (60µl x the number of different ELISAs to be run on each sample).

c. Flick out blocking buffer and pat plates dry
d. Pipette 50µl of sample/standard per well
e. Incubate for 3hrs (rm temp) to overnight (4ºC) in a humidified chamber, sealed with parafilm

4. Develop plates
    a. Wash 6x by immersion in ELISA wash and pat dry
    b. Dilute HRP-anti-isotypes 1:1000 in blocking buffer
    c. Add 50µl per well and incubate for 1hr at rm temp

    d. Wash 6x by immersion in ELISA wash and pat dry.
    e. Make up 10ml substrate per plate (10ml substrate buffer, 1mg ABTS, 5µl H2O2 – do not allow this to sit for more than about 10mins after the H2O2 has been added)
    f. Add 100µl substrate per well
    g. Incubate for at least 15mins then read A405 on ELISA reader