Protocol 502_Antigen-specific ELISA

Materials

1. 1xELISA plate per 8 samples tested.
2. Coating buffer: 0.1 M $NaHCO_3$ (3.18 g Na_2CO_3 + 5.88 g $NaHCO_3$ made up to 1000 ml with water).
3. Blocking buffer: 2% w/v BSA in PBS (10 g BSA made up to 500ml with PBS, filter-sterilised; store at 4°C, and only open in the laminar flow hood).
4. Washing buffer: 0.05% Tween 20 in PBS (5 ml Tween 20 in a 15 ml tube; add about 8ml water and mix well, then add it all to 10 liters of PBS)
5. Diluent: 0.5% BSA in PBS, sterile filtered, store at 4°C.
6. Substrate buffer: 0.1 M sodium phosphate monobasic NaH_2PO_4, pH 4 (13.8 g NaH_2PO_4 made up to 1000ml or 14.6 g sodium phosphate monobasic monohydrate $NaH_2PO_4.H_2O$). Per 10 ml (needed for 1 plate), add 1 mg ABTS (Boehringer) and 5 μl H_2O_2 just before use and mix.
7. Antibodies: serum from HA107 i.v. injected germ-free or HA107 gavaged B6 mice.

Method

1. Coat the plates the night before the experiment with 50μl/well of the HA107 cytoplasmic protein (concentration:10μg/ml). Wrap plates and incubate at 4°C overnight.
2. In the morning, flick off the coating solution into the sink, wash plates 4x by immersing in washing buffer and flicking the buffer from the plate into the sink. Blot the plates by tapping onto paper towelling.
3. Add 100 μl/well of Blocking buffer. Incubate at room temperature for 1-2 hours.
4. During the blocking stage, mark the plates with the samples that they will contain, and prepare serum samples.
   Serum samples: On a separate plate add A1-H1 with 87 μl of diluent. Add 60μl of diluent to all other wells (A2-H12). Add 3 μl of each serum sample into row 1 and do the titrations as 1/3. (keep the last column as blank)
5. Wash 4x.
6. Transfer 50 μl of the titration to the ELISA plate (using an 8-channel pipette), starting from column 12 to Column 1.
7. Incubate 90 min at RT.
8. Wash the plates 4x.
9. Add 100 μl of secondary antibody (Anti-mouse IgG γ chain specific; peroxidase conjugated A3673 Sigma, 1:1000) and incubate for 30-60 minutes at room temperature.
10. Wash 4x. Add 100 μl of substrate buffer and wait 30-60 min for the colour reaction to be optimal, then read at OD405nm in a 96 well plate reader.
11. Export data as X=lg[IgG Conc.], Y=OD405nm