ELISA Protocol
December 12, 2006

MATERIALS

Secondary Antibody

Peroxidase-conjugated, goat anti-mouse, IgG, whole molecule (Sigma #A4416)

OR for rat antibodies

Peroxidase-conjugated, goat anti-RAT, IgG, whole molecule (Sigma #A9037)

Substrate

3,3',5,5'-tetramethylbenzidine (TMB) solution (Vector Laboratories #SK4400)

Prepare according to the manufacturers directions: Add two drops "buffer pH 5.4", three drops of "substrate" and two drops of "hydrogen peroxide" for each 15mL of deionized water

0.1M Tris buffered saline (TBS)

50mM tris-HCl
100mM NaCl pH 7.5 in deionized water

Blocking solution

1% (w/v) instant non-fat dry milk (Carnation) In 0.1M TBS

Wash buffer

0.1% (w/v) instant non-fat dry milk (Carnation) In 0.1M TBS

Other reagents

0.5N sulfuric acid

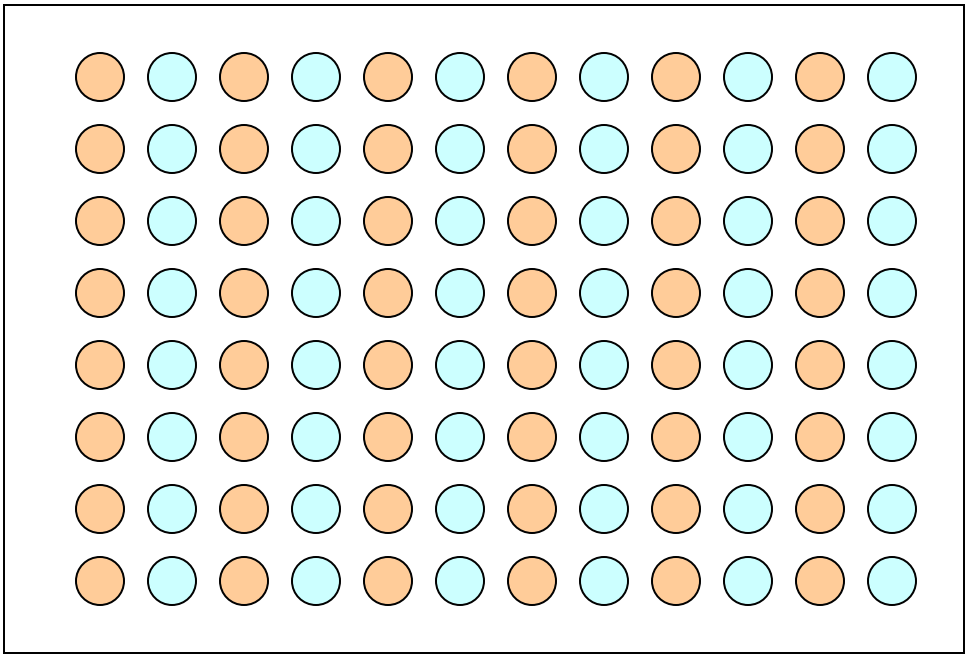
ELISA

1. Dissolve polysaccharide antigens in deionized water to a concentration of 10µg/mL.

2. Add 50µL of polysaccharide solution to each well in alternating columns. Add water containing no polysaccharide to the other columns as controls:

Sample wells: coat with polysaccharide in water

Control wells: coat with water



Place the coated plates (with lids removed) in an incubator at 37°C and allow them to concentrate to dryness overnight.

Dry plates can be stored covered at room temperature for 2 weeks.

3. Block wells with 200µL of Blocking solution for one hour at room temperature.

4. Remove blocking agent. Add 50µL of non-diluted hybridoma supernatant or diluted serum, incubate for one hour at room temperature.

5. Remove supernatant and wash wells three times each with 300µL/well (or 200µL if washing by hand) Wash buffer.

6. Add 50µL of peroxidase-conjugated secondary antibody diluted 1:5000 in Wash buffer and incubate for one hour at room temperature.

7. Remove secondary antibody and wash the wells five times with Wash buffer. Add 50µL of substrate solution.

8. After 20 minutes, stop the reaction by adding 50µL of 0.5N sulfuric acid to each well.

Do not remove the substrate solution or the acid!!

Read the optical density of the plate at 450 – 655nm.

Transfer the OD readings to Excel and subtract the reading of the control well from that of each test well on the same plate that contained the same primary and secondary antibodies but no immobilized polysaccharide.

Generate a bar graph that shows the absorbance of each test well (subtracted from its control) on the Y-axis (OD 0.0 – 1.0) and the various antibodies/dilutions on the X axis.