

## ELISA Protocol

### Coating Plates:

1. Make coating conjugate (IgG) to a working dilution. The last series of plates I coated, I used a dilution of 1:2000. Dilute conjugate with carbonate coating buffer. The dilution is made as follows:

conjugate:coating buffer

1:10 = 16 $\mu$ l: 144  $\mu$ l = 160  $\mu$ l

1:100 = 150  $\mu$ l: 1350  $\mu$ l = 1.5 ml

1:1000 = 1.1 ml: 9.9 ml = 11 ml

1:2000 = 10 ml: 20 ml = 30 ml

This is enough to coat 3 plates; you may adjust volumes accordingly for the number of plates you are running.

2. For multiple plates, identify each one by coloring in a letter on the left side of the plate with a sharpie. Make a plate map indicating the location of the samples.
3. Coat plates with 100  $\mu$ l coating conjugate/coating buffer.
4. Incubate at 4°C overnight.

### Detection:

1. Empty coating material and wash wells 2X with 200  $\mu$ l wash solution.
2. After second wash, empty plates and strike firmly on firm surface to remove any residual wash.
3. Block plates with 300  $\mu$ l blocking solution.
4. Incubate at room temp for 30-60 minutes.
5. Wash plates 2X as in step 1.
6. Add 100  $\mu$ l serum sample to plates with multichannel pipettor. Samples should be pre-aliquoted to eliminate time in aliquoting each sample individually.
7. Incubate at room temp. for 2 hrs., or 4°C overnight.
8. Wash 4-5X with wash sol'n.
9. Add 100 conjugate. Dilute conjugate in diluent to a final working concentration of 1:2000. Dilution for 3 plates may be done as follows:

conjugate:diluent

1:10 32  $\mu$ l: 128  $\mu$ l = 160  $\mu$ l

1:100 150 $\mu$ l: 1350  $\mu$ l = 1.5 ml

1:1000 1.1 ml: 9.9 ml = 11 ml

1:2000 10 ml: 20 ml = 30 ml

10. Incubate at room temp. for 2 hrs., or 4°C overnight.
11. Wash 4-5X with wash sol'n.
12. Make fresh substrate each time. 20 ml substrate buffer/ 1 pNPP tablet. If there is a significant amount of substrate buffer left, wrap conical in aluminum foil and store at -20°C. Keep in mind that substrate

should be at room temp. when applied to plate.

13. Incubate for 30 min in the dark.

14. Read plate.

\*Note: Reagents should be applied to plate at room temperature for optimum binding. Cold temperatures inhibit binding. Also, when pre-aliquoting samples, volume should be 5  $\mu$ l more than total volume to account for what is lost in transfer.