



Title: *Basic Enzyme-Linked Immunosorbent Assay (ELISA)*

No: RTLP-GL-EL-1

Location:
Old CCRC Tripp Lab

Approval Date:
10 September 2004

Supersedes Date:

Materials:

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|--------------------|----------------------------------|------------------------|------------------------------------|
| •Lab coat | •Phosphate-Buffered Saline (PBS) | •Multi-channel pipette | •3M H ₂ SO ₄ |
| •Gloves | •Powdered non-fat milk | •Pipetteman | •NaHCO ₃ |
| •ELISA microplates | •Antibodies: 1° & 2° | •Pipetteman tips | •OPD Tablets |
| •Plastic wrap | •37°C incubator | •HRP Developing kit | •ELISA plate reader |
| •Tween-20 | •Inactivated antigen | •4°C Cold Storage | |
| •Paper Towel | | | |

Procedure:

1. Dilute inactivated antigen in coating buffer (50 mM NaHCO₃, pH 9.6).
2. Coat an ELISA microplate using 50µl of diluted antigen/well.
3. Incubate the plate for 1 hour at 37°C, or overnight at 4°C (with plastic cover to reduce evaporation).
4. Discard antigen by flicking plate onto paper towel.
5. Wash plate with 1 X PBS pH7.4 + 0.05% Tween 20.
6. Repeat step 5, then blot dry on a paper towel.
7. To prevent non-specific binding of unbound protein binding sites add 100µL of 3 % powdered skim milk (diluted in wash buffer) to wells.
8. Let this blocking buffer sit on the plate for 45 minutes at room temperature.
9. Wash two times as outlined in step 5. Blot dry.
10. Set up for a 1:2 dilution series by putting 50µl of blocking buffer in A2-A12, B2-B12, etc for the remainder of the plate.

11. Dilute the primary antibody (1° Ab) in blocking buffer (1:10 good starting dilution) and add 100µl to wells of column 1. Remove 50µl with a multi-channel pipette and transfer to column # 2. Mix up and down 3-4 times with pipette and transfer 50ul to #3. Be extra careful not to get air bubbles in pipette tips when transferring to next well, as this will throw off dilutions. Continue this procedure through well # 10. Use wells in column 11 and 12 as positive and negative control wells. Incubate plate for 1 hour at room temperature.
12. Discard 1°Ab by flicking the plate onto paper towel, then wash plate three times as described in step 5.
13. Dilute conjugated secondary antibody (2° Ab) (see product insert for recommended ELISA dilution, normally 1:1000-2000) in blocking buffer and aliquot 50µl in each well.
14. Incubate for 1 hour at room temperature. Wash three times as described in step 5 and blot dry.
15. Add substrate (OPD (Sigma tablets) for HRP secondary Ab, mixed per package instructions), 50ul per well.
16. Let sit at room temperature until yellow color stops developing or 10-15 minutes.
17. Add 50ul of stop solution (3M H₂SO₄) to each well. Read plate at 490nm in ELISA plate reader.

Determine titer of samples as last dilution that is above mean + 3 standard deviation of negative control sera (normally diluted in column 12, A thru H).

Author	Management Approval/Date	Effective Date

