ELISA Protocol

Stamatatos Lab

Contact: Andrew Stuart (astuart@fredhutch.org)

General ELISA protocol for testing sera or antibodies against proteins.

Solutions Needed: 0.1M Sodium Bicarbonate (pH 9.4-9.6), Blocking Solution (1X PBS+10% Non-Fat Milk+0.3% Tween20), Dilution Buffer (1X PBS+10% Non-Fat Milk+0.03% Tween20), 1N Sulfuric Acid, Ammonium Thiocyanate.

1. Add 50ng of protein in 100ul sodium bicarbonate to each well of a high-binding 96 well plate. Incubate over night at room temperature.
2. Empty plates and then block by adding 150ul/well blocking solution, incubate 1 hour at 37^{o}C.
3. Wash plate, then serial dilute the antibody/sera in dilution buffer in the plate, incubate 1 hour at 37^{o}C.
4. Wash plate, add 100ul/well diluted HRP conjugated secondary antibody (1:3000 in dilution buffer), and incubate 1 hour at 37^{o}C.
5. Wash plate, add 50ul/well TMB substrate, and incubate 3 min at room temperature.
6. Stop development with addition of 50ul/well 1N sulfuric acid.
7. Immediately read plate (absorbance is measured at 450nm).

To determine the avidity: sera diluted to 1:500 is added to the blocked plate at step 3 and serially diluted ammonium thiocyanate (ranging from 10M to 1M) is then added to the diluted sera and incubated for 20 min at room temperature on a plate shaker