

## ELISA Protocol

### Buffers and Reagents:

**Coating buffer:** Bicarbonate/carbonate coating buffer (100 mM) or Phosphate buffer (100 mM)

Antigen should be diluted in coating buffer to immobilize them to the wells.

**Blocking solution:** Blocking agents that 1% BSA, eg. <http://www.fitzgerald-fii.com/bsa-reagent-grade-30-ab81.html> , Non-fat dry milk, serum are commonly used.

1% BSA/PBS-T or 0.25% Skimmed milk/PBS-T are usually used (TBS may also be used instead of PBS).

**Wash solution:** PBS-T (or TBS-T) PBS or Tris -buffered saline (pH 7.4) with 0.05% (v/v) Tween 20

PBS <http://www.fitzgerald-fii.com/pbs-10x-concentrate-85r-125.html>

**Reagent buffer:** Primary and secondary antibody should be diluted in blocking solution.

**Secondary antibody:** See our full list of conjugated secondary antibodies at <http://www.fitzgerald-fii.com/secondary-antibodies.html>, and choose by source animal, conjugated substance for your choice.

**Substrate solution:** TMB solution is commonly used substrate solution for HRP, such as those found at <http://www.fitzgerald-fii.com/catalogsearch/result/?q=TMB.html>

**Stop solution:** such as <http://www.fitzgerald-fii.com/stop-elution-solution-85r-123.html>

**Some other useful reagents:** <http://www.fitzgerald-fii.com/diluents.html>

### General Procedure:

#### Coating antigen to microplate

1. Dilute the antigen to a final concentration of 1 µg/ml (or optimized concentration for your antigen of choice) in coating buffer. Coat the wells of a PVC microtiter plate with the antigen by pipeting 50 µl of the antigen dilution.
2. Cover the plate with a plastic cover or film and incubate for 2 h at room temperature, or 4 deg C overnight.
3. Wash the plate 3 times by filling the wells with wash solution.  
The solutions are removed by flicking the plate over a sink. The remaining drops are removed by patting the plate on a paper towel.

#### Blocking

4. Fill the plate with blocking solution.
5. Cover the plate with a plastic cover or film and incubate over 30 minutes at room temperature, or 4 deg C overnight.
6. Wash the plate 3 times by filling the wells with wash solution.

The solutions are removed by flicking the plate over a sink. The remaining drops are removed by patting the plate on a paper towel.

### **Primary antibody**

7. The primary antibody is diluted to an optimized concentration in reagent buffer 50 µl of diluted solution is pipetted into the wells.
8. Cover the plate with a plastic cover or film and incubate for 1 h at room temperature, or 4 deg C overnight.
9. Wash the plate 3 times by filling the wells with wash solution.

The solutions are removed by flicking the plate over a sink. The remaining drops are removed by patting the plate on a paper towel.

### **Secondary antibody**

10. The secondary antibody is diluted to an optimal concentration in reagent buffer immediately before use.  
50 µl of solution is pipetted into the wells.
11. Cover the plate with a plastic cover or film and incubate for 1 h at room temperature, or 4 deg C overnight.
12. Wash the plate 3 times by filling the wells with wash solution.

The solutions are removed by flicking the plate over a sink. The remaining drops are removed by patting the plate on a paper towel.

### **Detection**

13. Dispense 100 µl of the substrate solution per well with a multichannel pipet.
14. After sufficient color development (if it is necessary) add 50 µl of stop solution to the wells.
15. Read the absorbance (optical density) of each well with a plate reader.