

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