ROCKLAND™
antibodies & assays

ELISA (enzyme linked immunosorbent assay)

The use of enzyme linked immunosorbent assay, or ELISA, provides an economical, rapid and highly sensitive method for screening a large number of samples. ELISA can be used to detect and quantitate peptides, proteins or antibodies. The assay is based upon an antigen-antibody interaction and subsequent enzymatic action on a substrate yielding a soluble colored product. Variations of the basic method exist for specialized applications. A basic method is outlined below.

Reagents and Equipment Required

- 96-well microtiter plate and microplate reader
- Bicarbonate Buffer. 50 mM NaHCO3 pH 9.5
- 45% (w/v) Fish Gel Concentrate.
- Phosphate Buffered Saline (PBS). Use 10X PBS, pH 7.2 (0.2 M Potassium Phosphate, 1.5 M NaCl) Code # MB-008. Dilute appropriate volume to 1X with deionized water.
- Blocking Solution. 3% (w/v) Fish Gel solution in PBS prepared by adding 3.33 g of 45% (w/v) Fish Gel to 50 ml of PBS.
- Antibody Diluent. 1% (w/v) Fish Gel solution in PBS prepared by adding 1.11 g of 45% (w/v) Fish Gel to 50 ml of PBS.
- NP-40 Solution. 0.05% (v/v) NP-40 solution prepared by adding 0.5 ml 10% NP-40 to 100 ml of PBS.
- Substrate Solution. Use ABTS (code ABTS-100) for peroxidase or pNPP (code NPP-10) for alkaline phosphatase.

Procedure

1. Prepare antigen at 5.0 ug/ml in carbonate buffer. Load 200 ul per well. Cover plate to minimize evaporation. Allow antigen to bind at 4° C. The time required to bind antigen varies and may range from a few hours to overnight. Remove antigen. Wash 3 times with PBS.
2. Add 300 ul blocking solution per well. Allow blocking to occur at room temperature for 2 hours. Remove blocking solution and wash 3 times with PBS.
3. Prepare serial dilutions of antibody to be titered in antibody diluent. Load 100 ul of diluted antibody per well. Allow the antibody to bind at room temperature for 1 h. Remove antibody and wash 3 times with NP-40 solution.
4. Prepare enzyme conjugated secondary antibody by dilution in antibody diluent. The optimum dilution may be lot specific. Refer to back cover inset for recommended dilution ranges. Load 100 ul of diluted enzyme conjugated antibody per well. Allow the conjugate to bind at room temperature for 30 minutes. Remove conjugate and wash 3 times with NP-40 solution.
5. Add 100 ul of desired substrate solution per well. Allow color to develop at room temperature for 30 minutes.
6. Read absorbances on plate using microplate reader.

Notes

It may be required to optimize the amount of a reagent in the assay by performing a checkerboard titration. This is accomplished by serial dilution of one reagent across the plate and serial dilution of the other reagent down the plate. This design permits you to analyze different concentrations of the two reagents in each well and to obtain the optimal combination of both reagents. It is important that the coating solution is absolutely free of detergents because competition for binding may cause low and/or uneven binding.

Excessive concentrations of coating protein may actually lead to less coating. Always use high quality antibody conjugates. For alkaline phosphatase conjugates replace PBS with TBS. The intensity of the resultant color produced when the substrate is added should correlate to the concentration of the primary antibody and the respective antigen.

References

Phelps, D.C., Nemec, S., Baker, R. and Mansell, R. (1990) Immunoassay for Napthazarin Phytotoxins Produced by Fusarium solani. Phytopathology 80; 298- 302.

Harlow, E. and Lane, D., Antibodies - A Laboratory Manual. Cold Spring Harbor Laboratory (1988).