ROCKLAND

Visit ModDetect Webpage

✔ PATENT PENDING

ModDetect™
ELISA Protocol

I. Reagents Required

Product	Preparation	Suggested Product(s)
96-well Microtiter Plate, 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 NaCl) Dilute appropriate volume to 1X with deionized water.	MB-008
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.	
PBST Solution	10X PBST (0.2 M Potassium Phosphate 1.5 M Sodium Chloride, 0.5% (v/v) Tween-20, pH 7.2)	MB-075-1000
Substrate Solution	Use TMB for peroxidase visualization.	TMBE-100

II. Indirect & Sandwich ELISA Concentrations

Indirect ELISA:
- 5 µg/mL BSA-conjugated oligo
- 5 µg/mL ModDetect primary antibody
- 1:8000 A-Ms secondary antibody (enzyme-conjugated)

Sandwich ELISA:
- 2 µg/well capture antibody
- 0.5 µg/mL detection antibody

III. Procedure

1. Prepare coating conditions, as listed above, in carbonate buffer. Load 100 µL per well. Cover plate to minimize evaporation. Allow to bind at 4°C. The time required to bind antigens varies and may range from a few hours to overnight. Remove antigen or excess capture antibody. Wash 3 times with PBS.
2. Add 200 µL blocking solution per well. Allow blocking to occur at room temperature for 1 hour. Remove blocking solution.
3. Prepare serial dilutions of antibody to be titered in blocking diluent, or serial dilutions of oligo target in blocking diluent if performing a sandwich ELISA. Load 100 µL of diluted antibody per well. Allow the antibody to bind at room temperature for 1 hour. Remove antibody and wash 3 times with PBST solution.
4. Prepare enzyme-conjugated secondary antibody, or enzyme-conjugated streptavidin (if needed) by dilution in antibody diluent. The optimum dilution may be lot-specific. Load 100 µL of diluted enzyme-conjugate per well. Allow the conjugate to bind at room temperature for 1 hour. Remove conjugate and wash 3 times with PBST solution.
5. Add 100 µL 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.