

## LP107 - INDIRECT ELISA

The following is a general protocol for an indirect ELISA. The precise conditions should be optimized for a particular assay. Expected OD490 readings are between 0.7 and 1.3 for a substrate development time of 20-30 minutes.

### Materials

Multi-pipette reservoirs  
1.5-ml microcentrifuge tubes  
96-well Nunc Immuno MaxiSorp microtiter plate

### Solutions

10 X TBS: 500 mM Tris, 1M NaCl pH7.4

1 X TBS: 50 ml 10X TBS, fill to 500 ml with distilled water. pH7.4

Coating Solution: 50 mM sodium carbonate, pH 9.6 is preferred. Alternatives include 1X PBS, 1X TBS depending on the pl of the protein or peptide used.

Blocking Solution: 1% RIA grade BSA (Sigma – A7888) in 1X TBS +0.05% Tween 20

Primary/Secondary Antibody Solution: 1X Blocking Solution

Wash Solution: 1X TBS + 0.05% Tween 20 (use 1X PBS + 0.05% Tween 20 if PBS used as a coating solution)

Citrate Buffer: 121.5 ml of 0.1M Citric Acid, pH to 5.0 using 128.5ml 0.2M dibasic sodium phosphate. Fill to 500ml with distilled water and check pH.

HRP Substrate Buffer: Prepare 10 ml HRP Substrate Buffer by mixing 9 ml citrate buffer, 1 ml 4 mg/ml OPD. Add 4 µl H<sub>2</sub>O<sub>2</sub> immediately before use.

Stop Solution: 4 N H<sub>2</sub>SO<sub>4</sub>

### Protocol

1. Dilute the peptide antigen to 2 µg/ml in Coating Solution.
2. Add 100 µl diluted antigen to appropriate wells. Cover with sealing tape and incubate at 4°C overnight. Alternatively, incubate at room temperature for 2 hours
3. Empty plate and tap out residual liquid.
4. Wash twice with 200 µl Wash Solution.
5. Add 200 µl Blocking Solution to each well. Incubate 2 hours at room temperature.
6. Empty plate and tap out residual liquid.



7. Wash twice with 200  $\mu$ l Wash Solution.
8. Add 100  $\mu$ l diluted primary antibody to each well. Incubate 2 hours at room temperature.
9. Empty plate, tap out residual liquid.
10. Wash with 300  $\mu$ l Wash Solution. Invert plate to empty, tap out residual liquid. Repeat 3 times.
11. Add 100  $\mu$ l diluted HRP-conjugated secondary antibody to each well. Incubate 1 hour at room temperature in the dark.
12. Empty plate, tap out residual liquid and wash as described in step 10.
13. Dispense 50  $\mu$ l HRP Substrate Solution per well. Develop the color for 2-30 minutes at room temperature in the dark.
14. Stop color development by adding 50  $\mu$ l of Stop Solution per well and immediately read at 492 nm in plate reader.