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 pI 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 H2O2 immediately before use.

Stop Solution: 4 N H2SO4

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 temperate 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 µl Wash Solution.
8. Add 100 µl diluted primary antibody to each well. Incubate 2 hours at room temperature.
9. Empty plate, tap out residual liquid.
10. Wash with 300 µl Wash Solution. Invert plate to empty, tap out residual liquid. Repeat 3 times.
11. Add 100 µl 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 µl 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 µl of Stop Solution per well and immediately read at 492 nm in plate reader.