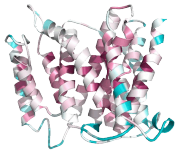
Direct ELISA Assay

Protocol



Prepare 250 ml of 50 mM sodium carbonate buffer at pH 9.0.

- Dissolve 1.32 g Na_2CO_3 in 150 ml DI water
- Adjust pH of soln to pH9.0
- Transfer to graduated cylinder and QS to 250ml. Ensure you rinse out the beaker you used.
- Store in a closed plastic bottle.

Prepare stop soln. (50 mM NaOH) – carefully mass about one gram of NaOH pellets. Calculate how much water to add. 1/4th of the mass of pellets to water. Store in plastic bottle, NOT glass.

Determine how much of antigen you will need to bind for that experiment. Each well will only need 50 µl. Make certain to allow for loss by making 1.2 to 2 times more than you need. Dilute your antigen with the carbonate buffer to a final concentration of 1 - 5 µg/ml. Tap or shake the plate to evenly distribute antigen.

Incubate overnight at 4° C with parafilm or wrap in plastic wrap to reduce evaporation. OR 2 hours at room temperature.

Fill the well of the plate with deionized water from a plastic squirt bottle and flick into the sink. With the plate turned upside down, slap the plate to remove the residual solution. Repeat Twice.

Add 200 µl/well of blocking buffer (PBS containing 1% BSA [square bottles from SantaCruz] and 0.02% sodium azide [Hagen 102 stock shelves]) to block non-specific protein binding. Incubate for 1-2 hours at room temperature, or overnight at 4° C.

Remove blocking buffer by flicking and slapping. Wash once with PBS with 0.02% sodium azide. Damp plates are usually stable in resealable plastic storage bags for 4 weeks at 4° C. Before using, remove residual solution by wrapping each plate in a large paper tissue and gently flicking it face down onto several paper towels.

Add test antibody samples and controls at 50 µl/well diluted in blocking buffer. Antibodies will be serially diluted for determining titer. Incubate 1 hour at room temperature.

Wash plates three times with PBS containing 0.05% Tween-20, removing excess liquid as above.

Add 50 µl/well of alkaline phosphatase conjugated secondary antibody (make certain you are using the appropriate antibody anti-mouse or anti-rabbit and that it is AP conjugated) diluted to 1:1000 in blocking buffer. Incubate 1 hour at room temperature.

Remove liquid in wells as above. Wash three times with PBS containing 0.05% Tween-20 and slap plate dry, removing liquid as above.

Substrate Preparation:

- Prepare substrate buffer: 10 mM Diethanolamine, 0.5 mM $MgCl_2$, pH 9.8
- Just prior to use, dissolve pNPP in substrate buffer to a final concentration of 1.0 mg/ml (i.e. 5 ml of substrate buffer per 5 mg tablet. Add 75 µl or 100 µl per well.

Alternative – Use commercially available PNPP soln. Read instruction or add 75µl per well.

Allow to develop for 10-20 minutes or until positive control reaches an OD 405 of about 1.0. May take up to an hour. Stop reaction by adding 50 µl of 0.5 M NaOH.