



The Power to Quantify

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ELISA Protocol



- Coat microtiter plates with target protein diluted in 50 mM carbonate buffer at pH 9.0. Optimal concentrations should be determined by titration, but for purified antigens 50 μ l per well at 1 μ g/ml is usually sufficient. Incubate overnight at 4° C covered with parafilm.
- Remove antigen solution. Add 200 μ l/well of blocking buffer (PBS containing 1% BSA and 0.02% azide) to block non-specific protein binding. Incubate for 1–2 hours at room temperature, or overnight at 4° C.
- Remove blocking buffer. Wash once with PBS with 0.02% azide. Damp strip wells or plates are usually stable in resealable plastic storage bags for 4 weeks at 4° C. Before using, remove excess liquid.
- Add test antibody samples and controls at 50 μ l/well diluted in blocking buffer. Antibodies may be serially diluted for determining titer or diluted to previously determined working concentration for screening assays or antigen quantitation. Incubate 1 hour at room temperature.
- Wash plates three times with PBS containing 0.05% Tween-20 (Tween-20: [sc-29113](#)), removing excess liquid as above.
- Add 50 μ l/well of alkaline phosphatase conjugated secondary antibody ([Conventional Secondary Antibodies for Western Blotting](#)) diluted to 1:100–1:1000 in blocking buffer. Optimal antibody concentration is determined by titration. Incubate 1 hour at room temperature.
- Remove liquid in wells. Wash three times with PBS containing 0.05% Tween-20 and slap plate dry.
- Wash wells once with diethanolamine buffer (10 mM diethanolamine, 0.5 mM MgCl₂ (pH 9.5) and remove liquid.
- Dilute substrate (PNPP, [sc-3720](#)) in diethanolamine buffer to a final concentration of 1 mg/ml. Add 50 μ l/well. Allow to develop for 10–20 minutes or until positive control reaches an OD 405/490 of about 1.0. Stop reaction by adding 50 μ l of 0.1 M EDTA (EDTA: [sc-29092](#)), pH 7.5. Read plates on microtiter plate reader at OD 405/490.