



PROCEDURE RECOMMENDATION: COMPETITIVE ELISA

This procedure has been developed for applications where the concentration of analyte must be determined in an aqueous sample. Immunoassay reagent pairs from SLRC have been optimized for this competitive ELISA procedure. Other reagents and supplies are standard for any ELISA procedure and are available from many commercial sources.

Reagents and Supplies:

Immunoassay Reagent Pair (Monoclonal Antibody and BSA conjugate)
Anti-mouse-Ig enzyme conjugate (e.g. Goat anti-mouse-Ig, alkaline phosphatase conjugate)
Substrate for enzyme conjugate (e.g. p-nitrophenyl phosphate for alkaline phosphatase) in appropriate buffer
PBS (0.15M NaCl, 20mM Phosphate, pH 7.5)
PBST (PBS with 0.05% Tween-20)
Microwell plates or strips (flat bottom, high protein binding, ELISA grade)
Microplate reader

Step 1 - Coating microtiter plates or strips. Dilute BSA conjugate in PBS [not PBST] according to specified dilution factor (usually 1500X). Distribute to microwells at 50 ul/well. Leave at room temperature for 1 hr or at 4 °C overnight.

Step 2 - Prepare samples and standards. Prepare a standard curve of your analyte in the concentration range 0 – 1000 ng/ml. Dilutions may be made in PBST or other suitable buffers. **IMPORTANT:** In order to interpret the assay, you must include a zero control with diluent alone. Samples may be diluted in PBST if the concentration of analyte is expected to be higher than 1000 ng/ml.

Step 3 – Prepare antibody. Dilute monoclonal antibody in PBST according to specified dilution factor (usually 1500X).

Step 4 – Antibody/Sample Incubation. Wash microwells thoroughly in PBST. Add 25 ul/well of sample/standard to wells, then add 25 ul/well of antibody to wells. Incubate 30 min. at room temperature.

[Note: for higher sensitivity, samples may be added at 45 ul/well, and antibody diluted 5X less than the specified dilution factor (i.e. 1:300 instead of 1:1500) and added at 5 ul/well. Alternatively, use a higher dilution of antibody (1:5,000 instead of 1:1500) – this will lower the signal but may also improve sensitivity in the lower range of analyte concentration.]

Step 5 – Secondary antibody. Wash microwells thoroughly in PBST. Add 50 ul/well of Goat anti-mouse-Ig enzyme conjugate, diluted in PBST according to supplier instructions. Incubate 30 min. at room temperature.

Step 6 – Substrate and Result. Wash microwells thoroughly in PBST. Add 50 ul/well of substrate. After sufficient development time, read absorbance at the appropriate wavelength.

Step 7 - Assay interpretation. Zero control wells have maximal absorbance. Samples with lower absorbance are positive for analyte. Make a standard curve (absorbance vs. concentration). Analyte concentration in samples may be calculated from the standard curve.