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General Protocol ELISA

Protocol may need local adjustments and/or according to kit-specific instructions

Materials/reagents

Coating antibody (1-10 µg/ml in coating buffer)
Samples, standards and controls
Primary antibody (unlabeled or biotinylated)
Enzyme labeled secondary antibody
Substrate
Coating buffer: 0.15 M sodium carbonate, 0.35 M sodium bicarbonate, pH 9.6 (Carbonate Coating Buffer)
Phosphate Buffered Saline (PBS), pH7.4
Blocking buffer: PBS, 1% BSA
Wash solution: PBS, 0.05% Tween-20
Dilution buffer: PBS, 0.05% Tween-20, 0.1% BSA; PBS, 0,1%BSA
Stop Solution; 2 % oxalic Acid

Procedure

Before the assay, both antibody preparations should be purified and albumin-free. The detection antibody must be labeled.

1. **Coating with capture antibody**
   Coat the wells by adding approximately 100 µl of coating antibody solution to each well. The amount of antibody used will depend on the individual assay, optimize the buffer and the coating concentration (1-10 µg/well).
   Aspirate the coating solution.

2. **Blocking**
   The remaining sites for protein binding on the microtiter plate must be saturated by incubating with blocking buffer. Fill the wells with 200 µl blocking buffer. Cover the plate with adhesive plastic and incubate for 60-90 minutes at room temperature or overnight at 4°C.
   Wash wells four times with wash solution.

3. **Sample incubation**
   Add 100 µl of the diluted samples, standards and controls to the wells. All dilutions should be done in the dilution buffer.
   Cover the plate with adhesive plastic and incubate for 60-90 minutes at 37°C.
   Wash the plate four times with wash solution.

4. **Incubation with primary antibody**
   Add 100 µl of the primary antibody. The amount to be added can be determined in preliminary experiments. For accurate quantification, the primary antibody should be used in excess. All dilutions should be done in the dilution buffer.

Cover the plate with adhesive plastic and incubate for 60-90 minutes at room temperature or at -20 °C.
Wash four times with wash solution.

5. **Incubation with the secondary antibody (or Streptavidine-HRP in case of biotinylated primary antibody)**
   Add 100 µl of the labeled secondary antibody. The amount to be added can be determined in preliminary experiments. For accurate quantification, the labeled secondary antibody should be used in excess. All dilutions should be done in the dilution buffer.
   Cover the plate with adhesive plastic and incubate for 60-90 minutes at room temperature or at -20 °C .
   Wash four times with wash solution.

6. **Substrate incubation**
   Add substrate as indicated by manufacturer.
   After suggested incubation time has elapsed, add the stop solution to each well.
   Optical densities at target wavelengths can be measured on an ELISA reader within thirty minutes after adding stop solution.

7. **Analysis of the data**
   Calculate the average absorbance values for each set of duplicate standards, samples and controls.
   If individual absorbance values differ by more than 15% from the corresponding mean value, the result is considered suspect and the sample should be re-assayed.
   Create a standard curve by reducing the data using computer software capable of generating a good curve fit.
   If the samples have been diluted, the concentration determined from the standard-curve must be multiplied by the dilution factor.