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UC Davis - ELISA Protocol

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Protocol status: Working

We use this protocol and it's working

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Abstract

Summary:

The ELISA (Enzyme Linked ImmunoSorbent Assay) is an assay method used for the quantification of various proteins. A plate is coated with a capture antibody specific to the analyte of interest. Standards and samples are pipetted into the plate. After an incubation period the wells are washed and any unbound analyte is removed. An enzyme-linked antibody that is specific to the analyte of interest is added to the wells. After an incubation period the wells are washed and any unbound antibody is removed. The analyte of interest is now sandwiched between the capture antibody and the enzyme-linked antibody. A substrate (normally 3,3',5,5'-tetramethylbenzidine) is added which reacts with the enzyme (normally horseradish peroxidase) conjugated to the secondary antibody. A blue color develops in proportion to the amount of analyte sandwiched between the two antibodies. After an incubation period the reaction is stopped with the addition of an acid which turns the blue color to yellow. The intensity of the color development is measured using a spectrophotometer. The absorbance readings and known concentrations of the standards are used to generate a standard curve, and the absorbance readings of the samples are used to interpolate quantitative concentrations for the analyte of interest from the standard curve.

Materials

Reagents and Materials:

- Standards
- Antibody coated microplate
- Wash solution
- Conjugated antibody
- Substrate solution
- Acid solution

Prepare all standards and reagents according to kit instructions.

Troubleshooting

Before start

IMPORTANT: *This is a generic ELISA protocol. The steps may be slightly different for each assay. Refer to kit instructions for each specific kit for correct volumes, number of washes, and incubation times.*



- 1 Follow kit instructions for preparing standards and all reagents.
- 2 Follow kit instructions for volumes and incubation times. Pipet standards and samples. Incubate.
- 3 Follow kit instructions for volumes and incubation times. Wash plate. Add secondary antibody. Incubate.
- 4 Follow kit instructions for volumes and incubation times. Wash plate. Add substrate. Incubate.
- 5 Follow kit instructions for volumes and incubation times. Add acid.
- 6 Read absorbances using a spectrophotometer. Calculate standard curve and unknown values.