Biology | Immunology

Enzyme Linked Immunosorbent Assay ELISA

General Aim

To detect and measure the concentration of an analyte (usually antibodies, antigens, hormones, proteins or peptides) in solutions.

Method

Sandwich ELISA

Learning Objectives (ILOs)

- To practice the steps of a successful sandwich ELISA.
- To explain the importance of the washing steps.

Theoretical Background/Context

- ELISA begins with a coating step, where the first layer either an antigen or an antibody is absorbed to a polystyrene 96 well plate. Coating is followed by blocking and detection steps and finally reading the results on ELISA reader.
- There are four basic ELISA formats, allowing for a certain amount of flexibility which can be adjusted based on the antibodies available, the results required, or the complexity of the samples:
  1. Direct ELISA.
  2. Indirect ELISA.
  3. Sandwich ELISA.
  4. Competition or Inhibition ELISA.
- ELISA is one of the most sensitive immunoassays available. The typical detection range for an ELISA is 0.1 to 1 fmole or 0.01 ng to 0.1 ng.

Principle of Work

- The Enzyme Linked Immunosorbent Assay (ELISA) is a common laboratory technique which is used to measure the concentration of the analyte (usually antibodies or antigens) in solutions.
- The steps of the ELISA result in a colored end product which correlates to the amount of the analytes present in the original sample.
- In this experiment we will be performing Sandwich ELISA in order to detect the presence of an antigen.
- Sandwich ELISA typically requires the use of matched antibody pairs, where each antibody is specific for different, non-overlapping parts of the antigen molecule. The first antibody termed the capture antibody is coated to the plate. Next, the analyte or sample solution is added to the well.
- A second antibody layer, the detection antibody, follows this step in order to measure the concentration of the analyte. If the detection antibody is conjugated to an enzyme, then the assay is called a direct sandwich ELISA. If the detection antibody is unlabeled, then a second detection antibody will be needed resulting in an indirect sandwich ELISA.