

Immunological techniques. ELISA and western blotting

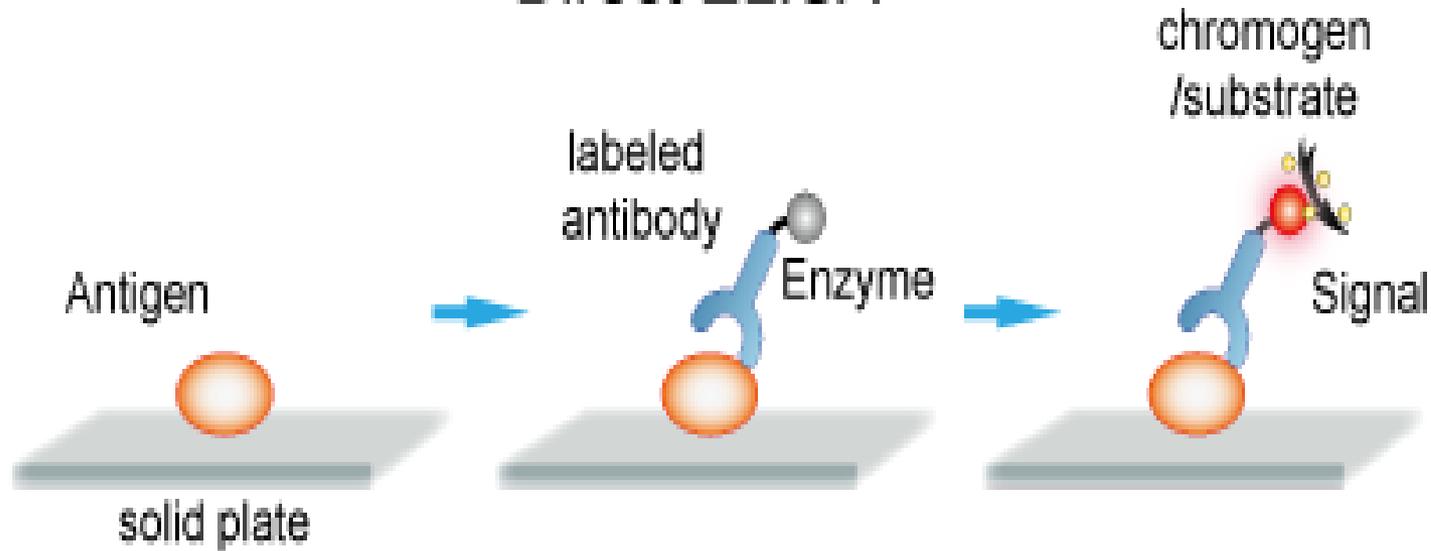
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ELISA (Enzyme-linked Immunosorbent Assay)

- ELISA (enzyme-linked immunosorbent assay) is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. Other names, such as enzyme immunoassay (EIA), are also used to describe the same technology. In an ELISA, an antigen must be immobilized on a solid surface and then complexed with an antibody that is linked to an enzyme. Detection is accomplished by assessing the conjugated enzyme activity via incubation with a substrate to produce a measureable product. **The most crucial element of the detection strategy is a highly specific antibody-antigen interaction.** It is performed in 96 well plate.

- Types of ELISA
- 1. Direct ELISA
- 2. Indirect ELIA
- 3. Sandwich ELISA
- 4. Competitive ELISA : The central event of competitive ELISA is a competitive binding process executed by original antigen (sample antigen) and add-in antigen (known antigen or target antigen).

Direct ELISA

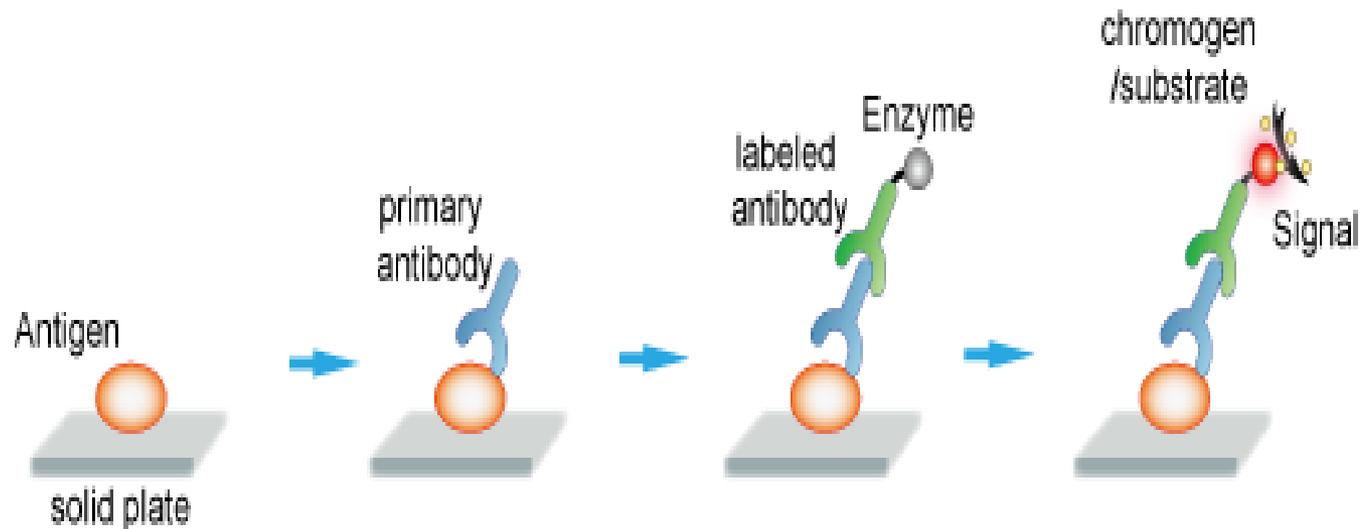


1. Antigen is coated onto wells by passive adsorption

2. Antibody conjugated with enzyme is added and incubated with antigen and incubation.

3. Substrate / chromophore is added and colour develops.

Indirect ELISA



1. Antigen is coated onto wells by passive adsorption and incubation.

2. Primary antibody is added and incubated with antigen.

3. Anti-species antibody conjugated with enzyme is added and incubated.

4. Substrate / chromophore is added and colour develops.

- Applications of ELISA
- Presence of antigen or the presence of antibody in a sample can be evaluated so can be useful for disease diagnosis.
- Determination of serum antibody concentrations in a virus test.
- Used in food industry when detecting potential food contaminants like antibiotics in shrimp.

- Applied in disease outbreaks- tracking the spread of disease e.g. HIV, bird flu, common, colds, cholera, STD etc.
- Detecting various hormones so can be used to detect maturity stages in fishes or other physiological conditions.
- In research; for detecting presence of antigens, enzymes, hormones, peptides etc.