

Lab. 5: Enzyme Linked Immunosorbent Assay ELISA

- ▶ Enzyme-linked immunosorbent assay (ELISA) test is the most widely used type of immunoassay.
- ▶ ELISA is a rapid test used for detecting or quantifying antibody (Ab) against viruses, bacteria and other materials or antigen (Ag).
- ▶ ELISA is so named because the test technique involves the use of an enzyme system and immunosorbent.
- ▶ It has now been widely applied in detection of a variety of antibody and antigens such as hormones, toxins, and viruses.

Materials needed in ELISA Testing

1. Pipettes, washer system, ELISA plate reader: Readers, washers and pipette are available as manual or automated system. One of the main factors affecting equipment selection is the number and types of test samples being run.

a. **ELISA Readers:** Readers need to have appropriate filter (650 nm and 450 nm).



b. **Pipette:** Are available as fixed as well as adjustable volume as well as single channel and multi-channel.

c. **Washing system:** It can be manual system that washes one row or column at a time or semi-automated systems that wash one strip or plate at a time or fully automated systems that can process multiple plates

2. Reagents needed for the testing– Concluded in the kit (coated plates, sample diluents, controls, wash concentrate, conjugate, substrate, stop solution)

- **Coated plates:** The **96-well plates** are made of polystyrene and are coated with either inactivated antigen or antibody. The function of the plate has to hold the immobilized either antigen or antibody. Antigen or antibody present in the sample will bind to the plate. This coating acts as the binding site for the antibodies or antigens in the sample.



- **Controls:** Negative and positive controls are provided in each kit. The controls help to normalize or standardize each plate. Controls are also used to validate the assay and to calculate sample results. Controls might be pre-diluted and ready to use. (Please refer to kit for specific instructions).

- **Conjugates:** ELISA conjugates are enzyme labeled antibodies that react specifically to plate bound sample analytes. Unbound conjugates are washed away after incubation and before the addition of substrate.

- **Wash Concentrate:** It acts as a buffered solution containing detergent to wash unbound material from the plate. (Not all test kits have wash concentrate; in that case distilled water can be used for washing; please refer to kit insert for specific instructions)

- **Stop solution:** It stops the enzyme substrate reaction and color development.

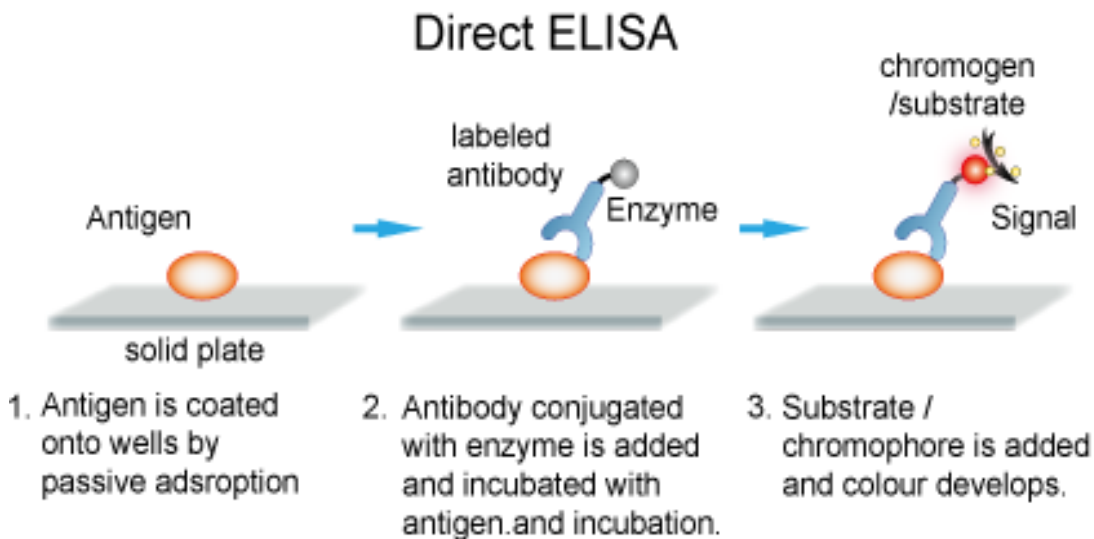
Types of ELISA

Frequently there are 3 types of ELISA on the basis of binding structure between the Antibody and Antigen.

1. Direct ELISA
2. Indirect ELISA
3. Sandwich ELISA
4. Competitive ELISA

1. Direct ELISA

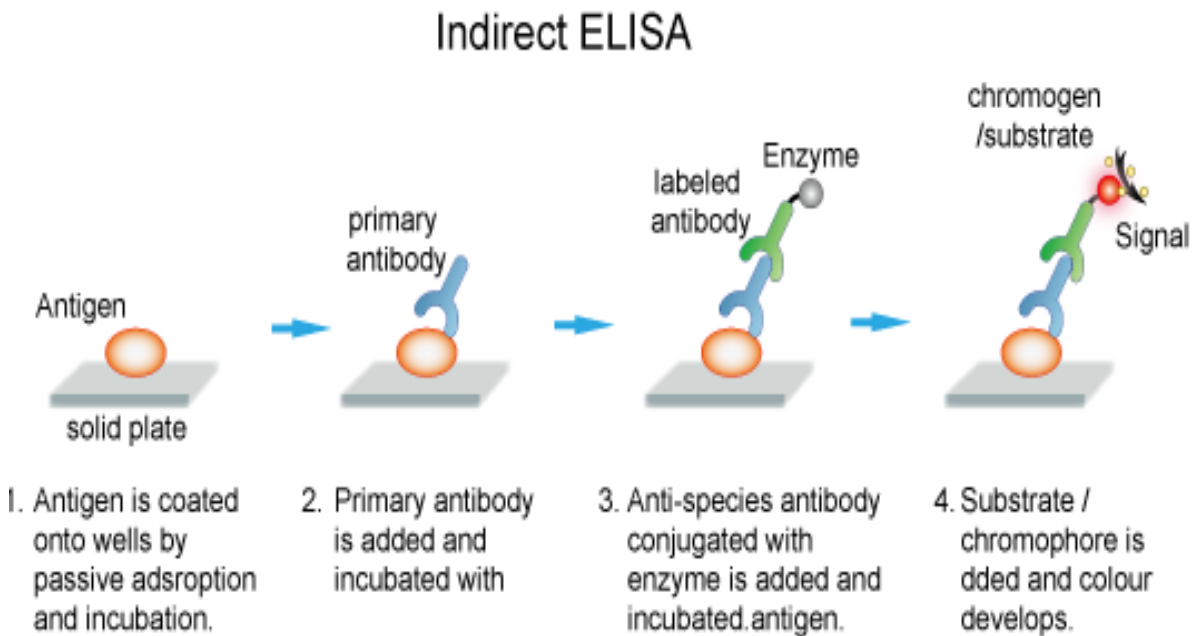
For direct detection, an antigen coated to a multi-well plate is detected by an antibody that has been directly conjugated to an enzyme. This detection method is a good option if there are no commercially available ELISA kits for your target protein.



2. Indirect ELISA

Antibody can be detected or quantitatively determined by indirect ELISA. In this technique, antigen is coated on the micro titter well. Serum or some other sample

containing primary antibody is added to the microtiter well and allowed to react with the coated antigen. Any free primary antibody is washed away and the bound antibody to the antigen is detected by adding an enzyme conjugated secondary antibody that binds to the primary antibody. Unbound secondary antibody is then washed away and a specific substrate for the enzyme is added. Enzyme hydrolyzes the substrate to form colored products. The amount of colored end product is measured by spectrophotometric plate readers that can measure the absorbance of all the wells of 96-well plate.



Application of ELISA

1. Presence of antigen or the presence of antibody in a sample can be evaluated.
2. Determination of serum antibody concentrations in a virus test.
3. Used in food industry when detecting potential food allergens.
4. Applied in disease outbreaks- tracking the spread of disease e.g. HIV, bird flu, common, colds, cholera, STD etc.