Enzyme-linked immunosorbent assay (ELISA)

The **Enzyme-linked immunosorbent assay (ELISA)** is an immunological assay commonly used to measure **antibodies, antigens, proteins and glycoproteins** in biological samples. Some examples include:

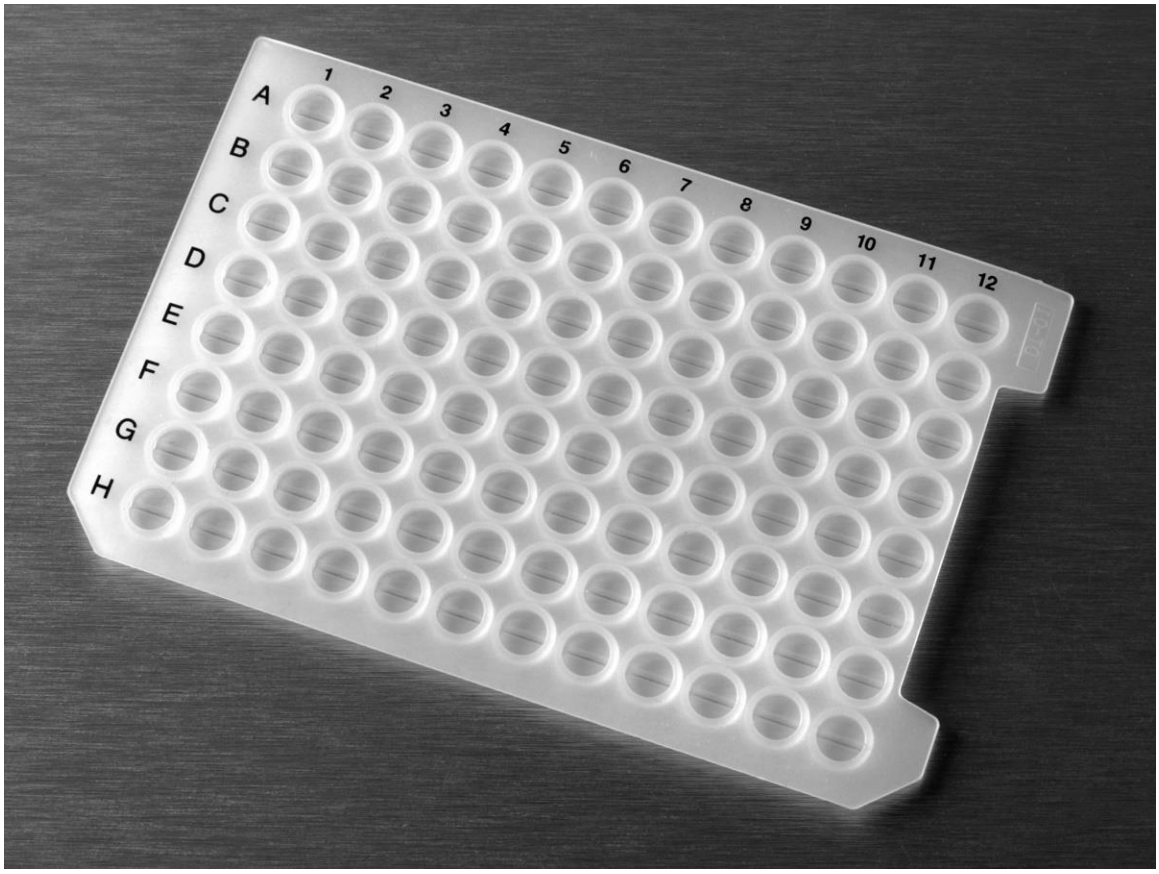
1. Diagnosis of HIV infection
2. Pregnancy tests
3. Measurement of cytokines or soluble receptors in cell supernatant or serum

Antibody: (also known as immunoglobulins abbreviated Ig) are gamma globulin proteins that are found in blood and are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses.

Antigen: The antigen is a substance that when introduced into the body stimulates the production of an antibody.

Immunoassay: A laboratory technique that makes use of the binding between an antigen and its homologous antibody to identify and quantify the specific antigen or antibody in a sample.

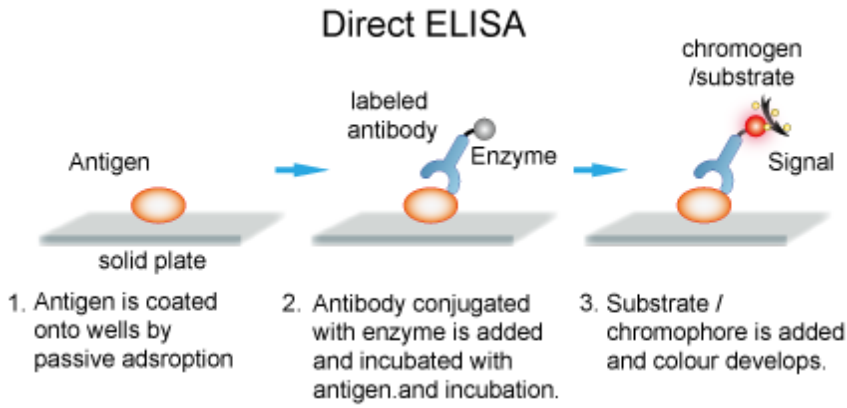
ELISA assays are generally carried out in 96 well plates, allowing multiple samples to be measured in a single experiment. These plates need to be special absorbent plates (e.g. NUNC Immuno plates) to ensure the antibody or antigen sticks to the surface. Each ELISA measures a specific antigen, and kits for a variety of antigens are widely available.



There are four major types of ELISA:

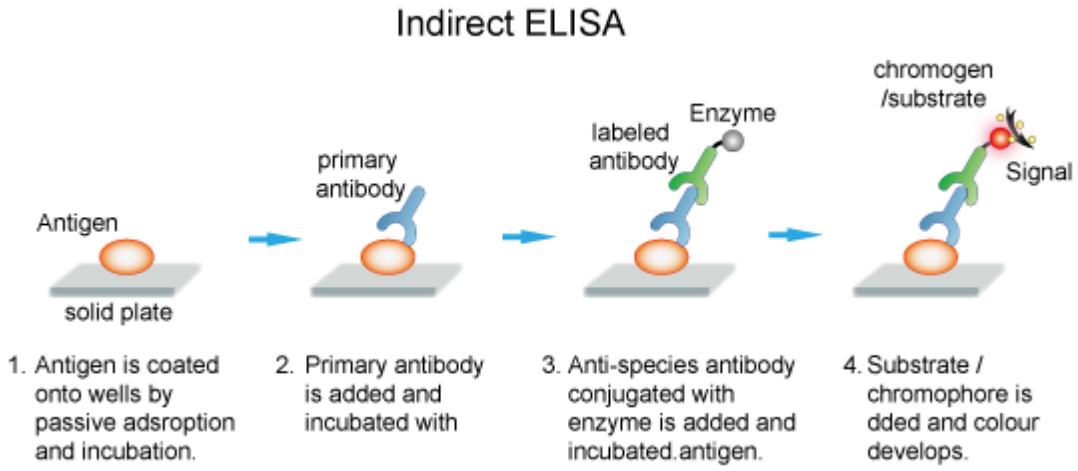
1. **Direct ELISA** (antigen-coated plate; screening antibody)

   In a direct ELISA, the antigen is immobilized to the surface of the multi-well plate and detected with an antibody specific for the antigen The antibody is directly conjugated to HRP or other detection molecules.



2. **Indirect ELISA** (antigen-coated plate; screening antigen/antibody)

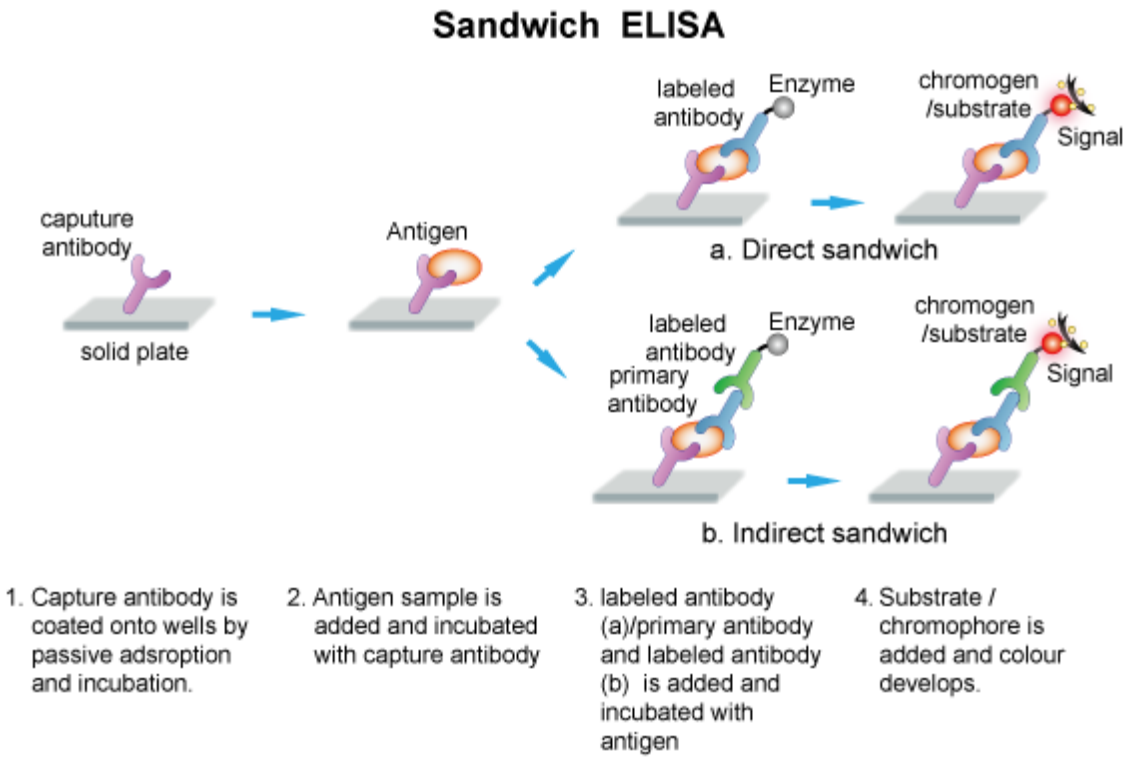
   It is a technique that uses a two-step process for detection, whereby a primary antibody specific for the antigen binds to the target, and a labeled secondary antibody against the host species of the primary antibody binds to the primary antibody for detection. As for direct ELISA assays, the antigen is immobilized to the surface of the multi-well plate.



3. **Sandwich ELISA** (antibody-coated plate; screening antigen)

The ELISA pictured in Figure 3 is what is known as a sandwich ELISA, here two sets of antibodies are used to detect secreted products, e.g. cytokines. The method is stepwise in the order shown. The 1st step is to coat the ELISA plate with capture antibody, any excess unbound antibody is then washed from the plate. The capture antibody is an antibody raised against the antigen of interest. Next, the sample (e.g. urine, serum, or cell supernatant) is added. Any antigen found in the sample will bind to the capture antibody already coating the plate. Samples are usually added in duplicate or triplicate (to allow for statistical analysis), and in varying concentrations to guarantee it falls within the levels of detection of the assay. Again any excess sample is washed from the plate.

In step 3, a detection antibody is added. This antibody is labelled with an enzyme, usually horse radish peroxidase or alkaline phosphatase. Detection antibody binds to any target antigen already bound to the plate. Finally, a substrate is added to the plate. ELISA assays are usually chromogenic using a reaction that converts the substrate (e.g. TMB or ABTS) into a coloured product which can be measured using a plate reader.



Determination of antigen concentration in a sample requires the production of a standard curve using antigens of a known concentration shown in (Figure 2). The concentration of antigen in a sample can then be calculated using the optical density (OD).

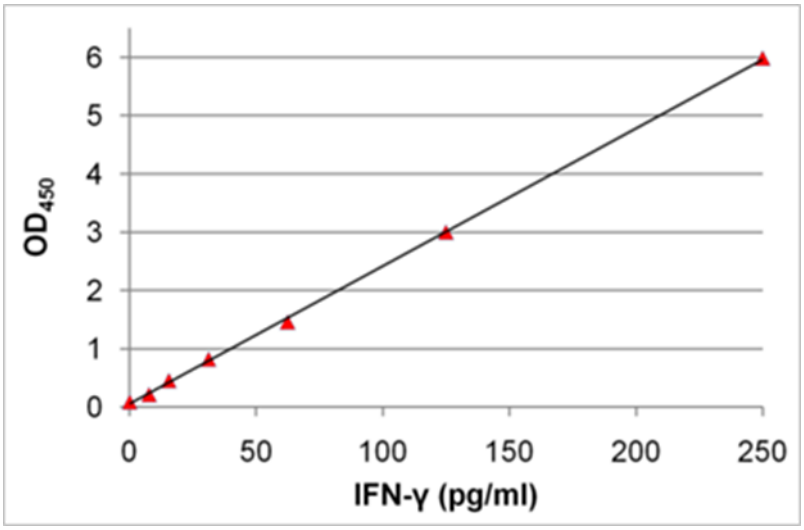


Figure 2. A typical standard curve. Shown is a standard curve for an IFN-γ ELISA. To work out the concentration of antigen in a sample, a standard curve using a solution of known concentration needs to be prepared.

4. **Competitive ELISA** (screening antibody)

   Also known as inhibition ELISA or competitive immunoassay, competitive ELISA assays measure the concentration of an antigen by detection of signal interference.