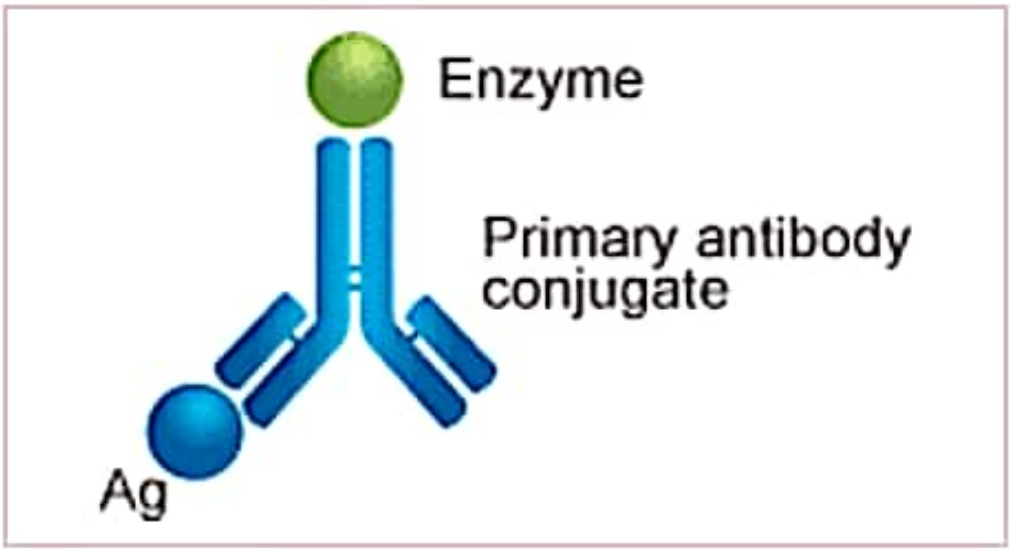
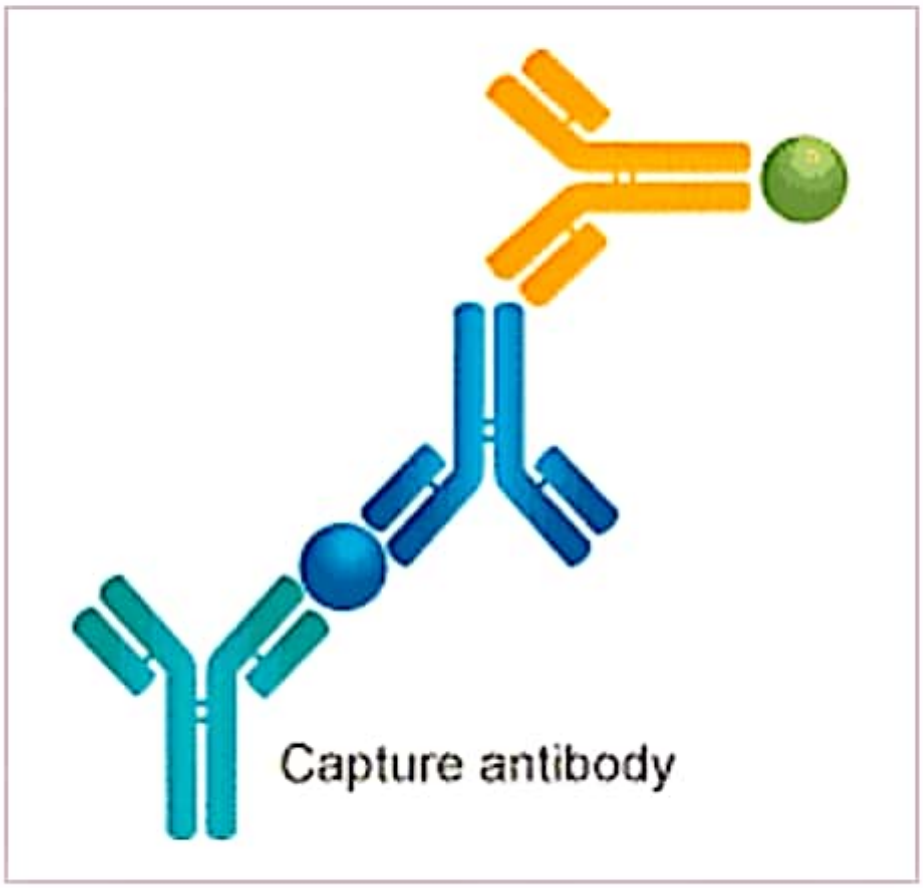
Description: An enzyme-linked immunosorbent **assay** (**ELISA**) is used to detect the presence of an antigen in a sample. The antigen is immobilized to the well of a plate by adsorption, or captured with a bound, antigen-specific antibody. A detection antibody is then added forming a complex with the antigen, if present.

Direct ELISA



In direct ELISA, only an enzyme-labeled primary antibody is used, meaning that secondary antibodies are not needed. The enzyme-labeled primary antibody "directly" binds to the target (antigen) that is immobilized to the plate (solid surface). Next, the enzyme linked to the primary antibody reacts with its substrate to produce a visible signal that can be measured. In this way, the antigen of interest is detected.

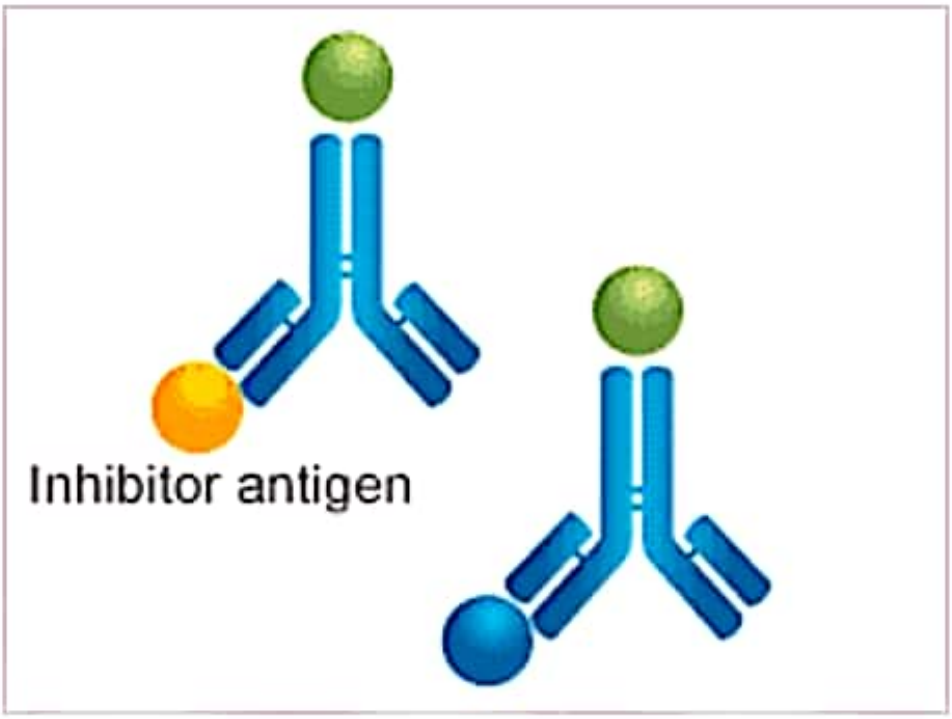
Sandwich ELISA



In direct and indirect ELISA, it is the antigen that is immobilized to the plate. In sandwich ELISA, however, it is the antibody that is immobilized to the plate, and this antibody is called capture antibody. In addition to capture antibody, sandwich ELISA also involves the use of detection antibodies, which generally include the unlabeled primary detection antibody and the enzyme-labeled secondary detection antibody.

Firstly, the antigen of interest binds to the capture antibody immobilized to the plate. Secondly, the primary detection antibody binds to the antigen. Thirdly, the secondary detection antibody binds to the primary detection antibody, and then the enzyme reacts with its substrate to produce a visible signal that can be measured.

Competitive ELISA



Compared with the three ELISA types above, competitive ELISA is relatively complex because it involves the use of inhibitor antigen, so competitive ELISA is also known as inhibition ELISA. In fact, each of the three formats, direct, indirect, and sandwich, can be adapted to the competitive format. In competitive ELISA, the inhibitor antigen and the antigen of interest compete for binding to the primary antibody. Here is a procedure of competitive ELISA:

Firstly, the unlabeled primary antibody is incubated with the sample containing the antigen of interest, leading to the formation of antigen-antibody complex (Ag-Ab). In this step, the antibody is excessive compared with the antigen, so there are free antibodies left.

Secondly, the Ag-Ab mixture is added to the plate coated with inhibitor antigen that can also bind to the primary antibody. The free primary antibody in the mixture binds to the inhibitor antigen on the plate, while the Ag-Ab complexes in the mixture do not and are therefore washed off.

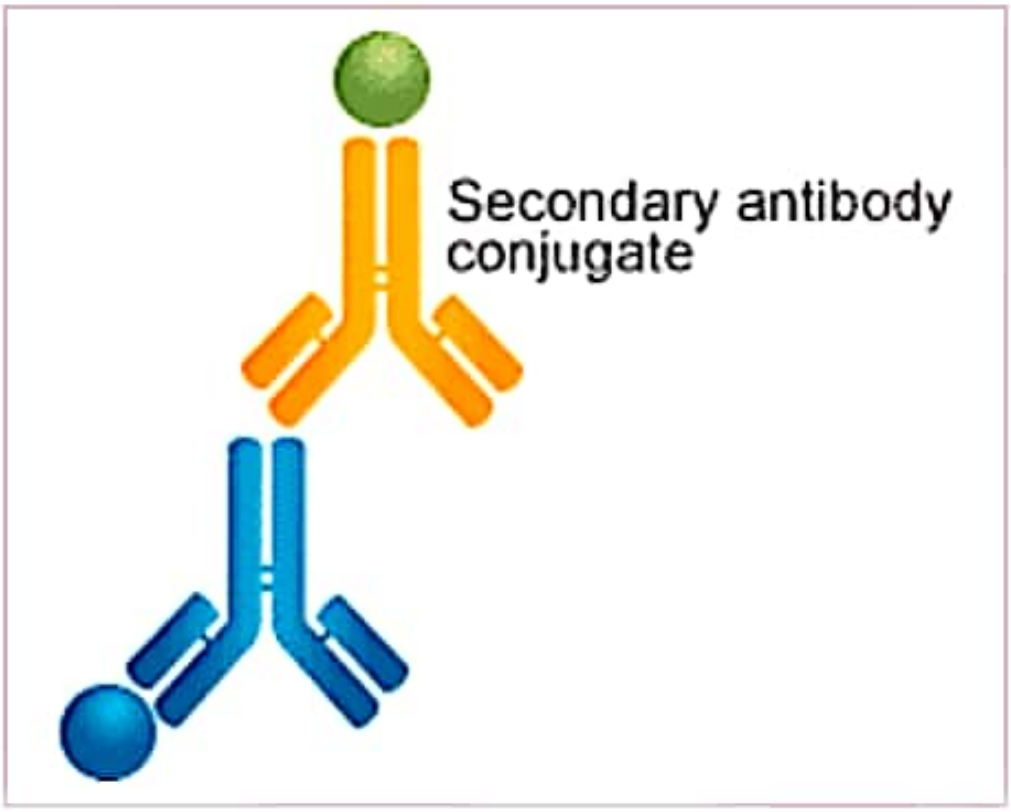
Thirdly, the enzyme-labeled secondary antibody is added to the plate and binds to the primary antibody bound to the inhibitor antigen on the plate.

Finally, a substrate is added to react with the enzyme and emit a visible signal for detection.

Through this procedure, you may find that the final signal is inversely associated with the amount of the antigen of interest in the sample, meaning that the more antigen in the sample, the weaker the final signal. This is because primary antibodies bound to sample antigen will be washed off, while free primary antibodies left will be captured by inhibitor antigen immobilized to the plate and be measured by an enzymatic reaction.

Competitive ELISA described here is based on antibody capture, in which the plate is coated with antigen. There is another type of competitive ELISA that is based on antigen capture, in which the plate is coated with unlabeled antibody. Furthermore, competitive ELISA generally uses a labeled antibody for detection, but sometimes it uses labeled antigen instead of a labeled antibody.

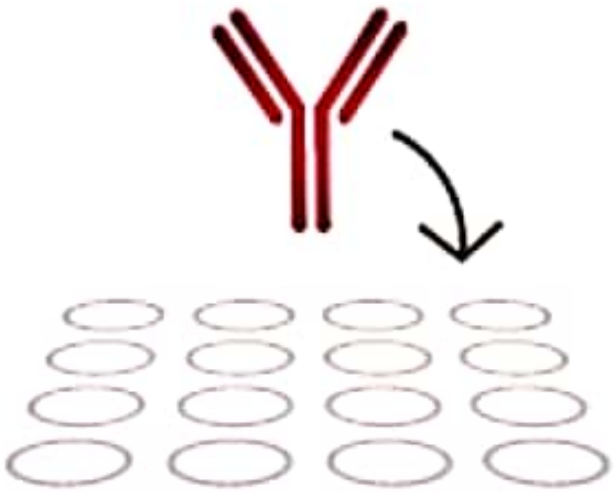
Indirect ELISA



In indirect ELISA, both a primary antibody and a secondary antibody are used. But in this case, the primary antibody is not labeled with an enzyme. Instead, the secondary antibody is labeled with an enzyme.

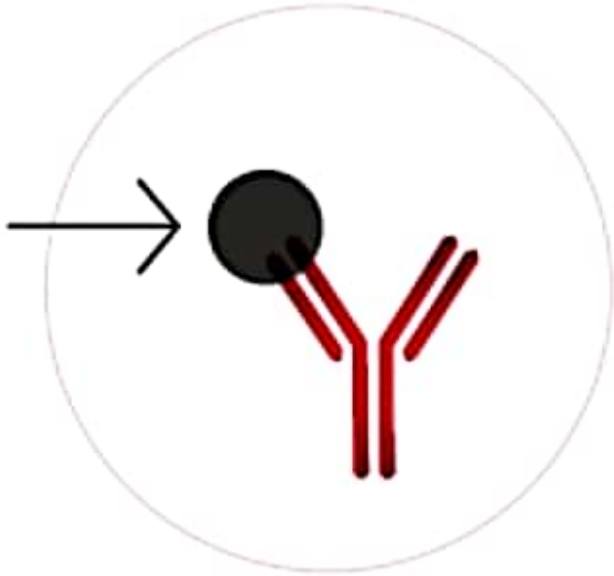
The primary antibody binds to the antigen immobilized to the plate, and then the enzyme-labeled secondary antibody binds to the primary antibody. Finally, the enzyme linked to the secondary antibody reacts with its substrate to produce a visible signal that can be measured.

ELISA Step-by-step



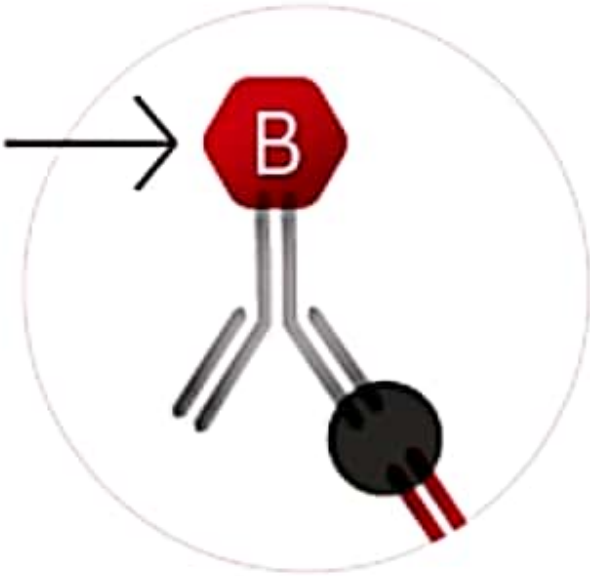
1. Antibody coating

Specific capture antibody is immobilized on high protein-binding plates by overnight incubation. Plates are blocked with irrelevant protein e.g. albumin.



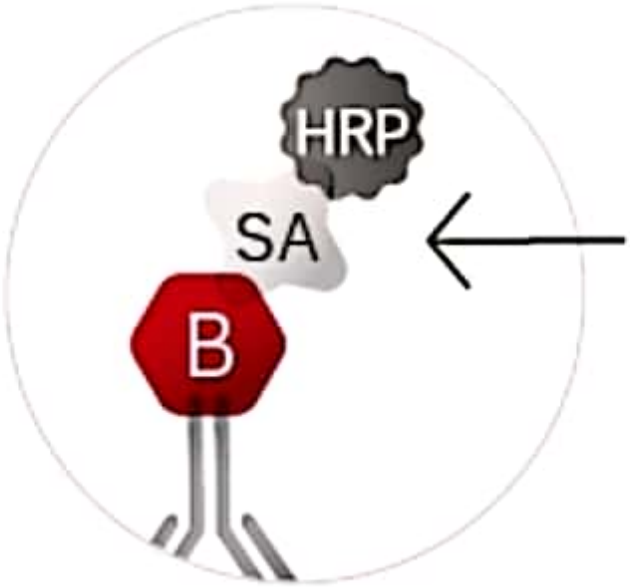
2. Protein capture

Samples and standard dilutions are added to the wells and will be captured by the bound antibodies.



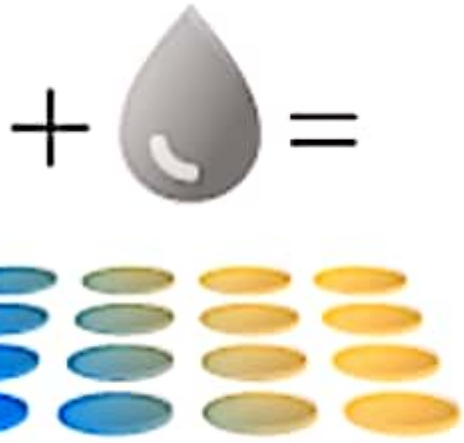
3. Detection antibody

Specific biotinylated detection antibody is added to the wells to enable detection of the captured protein.



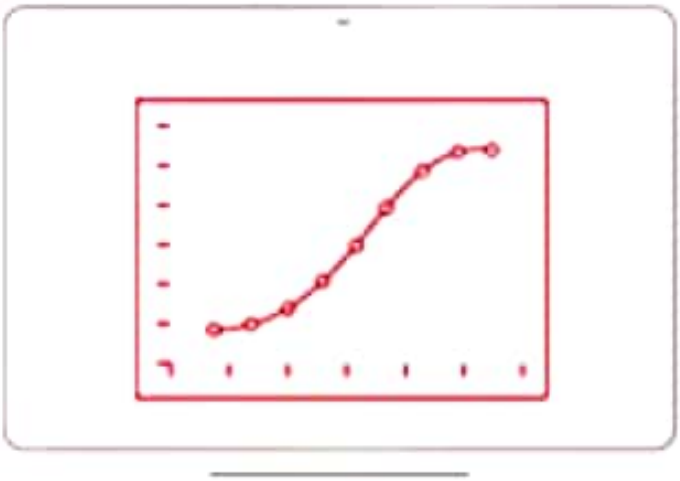
4. Streptavidin-enzyme conjugate

Streptavidin conjugated with alkaline phosphatase or horseradish peroxidase is added to the wells and will bind to the biotinylated antibody. Learn more about enzymes and substrates and their activities



5. Addition of substrate

Colorimetric substrate is added to the wells and will form a colored solution when catalyzed by the enzyme.



6. Analysis

Absorbance is measured in an ELISA reader and the amount of protein in the samples is determined.

Applications of ELISA

- Serum Antibody Concentrations
- Detecting potential food allergens
  - (milk, peanuts, walnuts, almonds and eggs)
- Disease outbreaks- tracking the spread of disease
  - e.g. HIV, bird flu, common, colds, cholera, STD etc
- Detections of antigens
  - e.g. pregnancy hormones, drug allergen, GMO, mad cow disease
- Detection of antibodies in blood sample for past exposure to disease
  - e.g. Lyme Disease, trichinosis, HIV, bird flu