

## **Principle of ELISA**

The principle of ELISA (Enzyme-Linked Immunosorbent Assay) is a widely used technique for the detection and quantification of specific proteins, antibodies, or antigens in a sample. ELISA is based on the specific binding between an antigen and an antibody, and it utilizes enzyme-conjugated antibodies and colorimetric or fluorescent substrates to generate a measurable signal. Here is an outline of the principle:

**Antigen or antibody immobilization:** The target antigen or antibody is immobilized on a solid surface, such as a microplate well, through non-covalent or covalent attachment. The immobilization can be achieved by direct adsorption, passive binding, or using capture antibodies specific to the target antigen or antibody.

**Blocking:** The solid surface is incubated with a blocking solution, typically containing a protein-based blocking agent (e.g., bovine serum albumin or non-fat dry milk). The blocking step helps to prevent non-specific binding and reduce background noise.

**Sample incubation:** The sample containing the unknown concentration of the target antigen or antibody is added to the well and allowed to incubate. If the antigen is being detected, the sample may contain the target antigen itself or be derived from a source containing the antigen. If the antibody is being detected, the sample may contain the target antibody or be derived from a source containing the antibody.

**Primary antibody binding:** After sample incubation, a primary antibody specific to the target antigen or antibody is added to the well. The primary antibody binds to the target antigen or antibody present in the sample.

**Washing:** The well is washed to remove any unbound or non-specifically bound molecules, including unbound sample components and excess primary antibodies.

**Secondary antibody binding:** An enzyme-conjugated secondary antibody, specific to the primary antibody species, is added to the well. The secondary antibody binds to the primary antibody, creating a sandwich complex if the target antigen or antibody is present.

**Washing:** The well is washed again to remove any unbound or non-specifically bound secondary antibodies.

**Enzyme substrate addition:** A colorimetric or fluorescent substrate is added to the well, which is converted by the enzyme conjugated to the secondary antibody into a detectable signal. The choice of substrate depends on the enzyme used, such as horseradish peroxidase (HRP) for colorimetric detection or alkaline phosphatase (AP) for colorimetric or fluorescent detection.

**Signal measurement:** The signal generated by the enzyme-substrate reaction is measured using a spectrophotometer for colorimetric detection or a fluorescence reader for fluorescent detection. The intensity of the signal is directly proportional to the concentration of the target antigen or antibody in the sample.

**Data analysis:** The obtained signal is compared to a standard curve generated from known concentrations of the target antigen or antibody to determine the concentration of the target in the sample. Alternatively, the signal can be analyzed qualitatively by comparing it to a cutoff value to determine the presence or absence of the target.

ELISA is a versatile and sensitive technique used in various research areas, including immunology, diagnostics, and biotechnology. It enables the detection and quantification of specific antigens or antibodies in a wide range of samples, such as serum, plasma, cell culture supernatants, or environmental samples.