ELISA

The enzyme-linked immunosorbent assay (ELISA) is an analytical biochemistry assay, which uses a solid-phase enzyme immunoassay (EIA) to detect the presence of a ligand (commonly a protein) in a liquid sample. The technique uses antibodies directed against the protein to be measured. ELISA has been used as a diagnostic tool in medicine, pathology, and biotechnology, as well as a quality control check in various industries.

In the most simple form of an ELISA, antigens from the sample are attached to a surface. Then, a matching antibody is applied over the surface so it can bind to the antigen. This antibody is linked to an enzyme activity, and in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change.

Types of ELISA

There are many types of ELISA tests for particular molecules that use the matching antibodies. ELISA tests are divided into several types based on how the analytes and antibodies are bonded and used. The major types are described below:

Direct ELISA

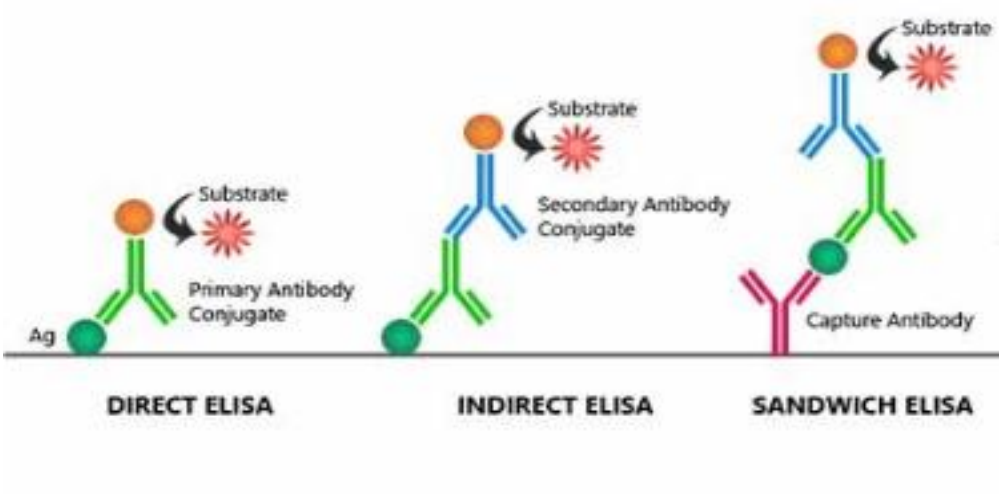
(1)A buffered solution of the antigen to be tested for is added to each well (usually 96-well plates) of a microtiter plate, where it is given time to adhere to the plastic through charge interactions. (2)A solution of nonreacting protein, such as bovine serum albumin or casein, is added to each well in order to cover any plastic surface in the well which remains uncoated by the antigen. (3) The primary antibody with an attached (conjugated) enzyme is added, which binds specifically to the test antigen coating the well. (4) A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme. (5) The higher the concentration of the primary antibody present in the serum, the stronger the color change. Often, a spectrometer or ELISA reader is used to give quantitative values for color strength.

Indirect ELISA

(1) Plate is coated with the antigen to be tested; (2The primary antibody is added, which binds specifically to the test antigen coating the well; (3) The secondary antibody with an attached (conjugated) enzyme is added, which binds specifically to the Fc region of the primary antibody; (4) substrate is added, and is converted by enzyme to detectable form.

Sandwich ELISA

(1) Plate is coated with a capture antibody; (2) sample is added, and any antigen present binds to capture antibody; (3) detecting antibody is added, and binds to antigen; (4) enzyme-linked secondary antibody is added, and binds to detecting antibody; (5) substrate is added, and is converted by enzyme to detectable form.


Substrate
Primary Antibody
Conjugate
Ag
Substrate
Secondary Antibody
Conjugate
Substrate
Capture Antibody
DIRECT ELISA
INDIRECT ELISA
SANDWICH ELISA

RADIOIMMUNOASSAY

A RIA is a very sensitive in vitro assay technique used to measure concentrations of substances, usually measuring antigen concentrations (for example, hormone levels in blood) by use of antibodies. Radioimmunoassay (RIA) uses radiolabeled molecules in a stepwise formation of immune complexes..

Although the RIA technique is extremely sensitive and extremely specific, requiring specialized equipment, it remains among the least expensive methods to perform such measurements. It requires special precautions and licensing, since radioactive substances are used.

The Method

Classically, to perform a radioimmunoassay, a known quantity of an antigen is made radioactive, frequently by labeling it with gamma-radioactive isotopes of iodine, such as 125-I, attached to tyrosine. This radiolabeled antigen is then mixed with a known amount of antibody for that antigen, and as a result, the two specifically bind to one another. Then, a sample of serum from a patient containing an unknown quantity of that same antigen is added. This causes the unlabeled (or "cold") antigen from the serum to compete with the radiolabeled antigen ("hot") for antibody binding sites. As the concentration of "cold" antigen is increased, more of it binds to the antibody, displacing the radiolabeled variant, and reducing the ratio of antibody-bound radiolabeled antigen to free radiolabeled antigen. The bound antigens are then separated and the radioactivity of the free (unbound) antigen remaining in the supernatant is measured using a gamma counter.