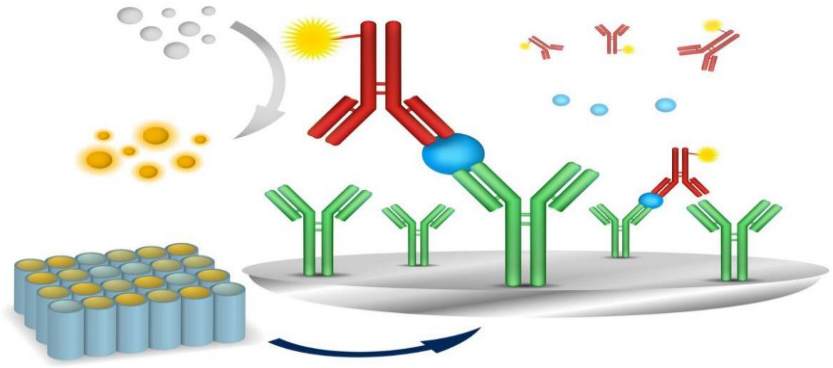
Lab.3



ELISA

ELISA (enzyme-linked immunosorbent assay) is a plate-based assay technique designed for detecting and quantifying peptides, proteins, antibodies and hormones.

Principle

ELISAs are typically performed in 96-well (or 384-well) polystyrene plates, which will passively bind antibodies and proteins. It is this binding and immobilization of reagents that makes ELISAs so easy to design and perform. Having the reactants of the ELISA immobilized to the microplate surface makes it easy to separate bound from non-bound material during the assay. This ability to wash away nonspecifically bound materials makes the ELISA a powerful tool for measuring specific analytes within a crude preparation.

ELISA Types

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

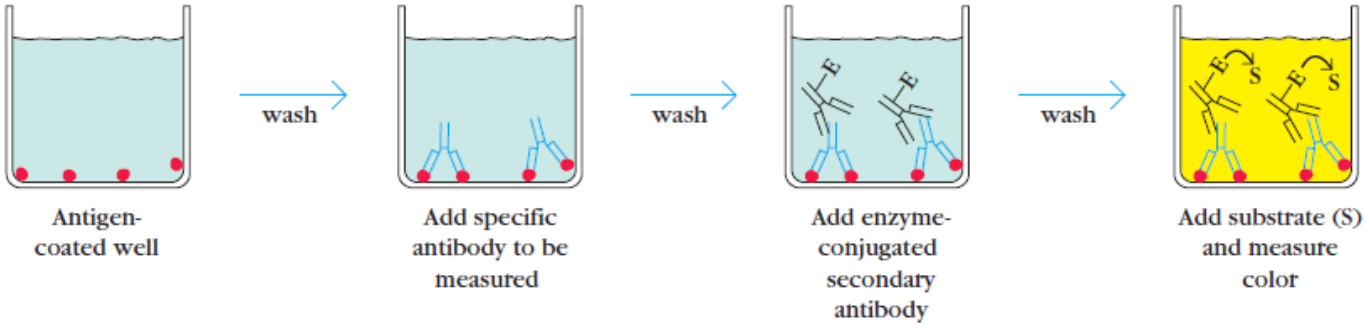
1. Indirect ELISA

2. Sandwich ELISA

3. Competitive ELISA

1. Indirect ELISA

Antibody can be detected or quantitatively determined by indirect ELISA. In this technique, antigen is coated on the microtiter 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.



Procedure of Indirect ELISA

1. Coat the micro titer plate wells with antigen.

2. Block all unbound sites to prevent false positive results.

3. Add sample containing antibody (e.g. rabbit monoclonal antibody) to the wells and incubate the plate at 37°c.

4. Wash the plate, so that unbound antibody is removed.

5. Add secondary antibody conjugated to an enzyme (e.g. anti- mouse IgG).

6. Wash the plate, so that unbound enzyme-linked antibodies are removed.

7. Add substrate which is converted by the enzyme to produce a colored product.

8. Reaction of a substrate with the enzyme to produce a colored product.

Advantages

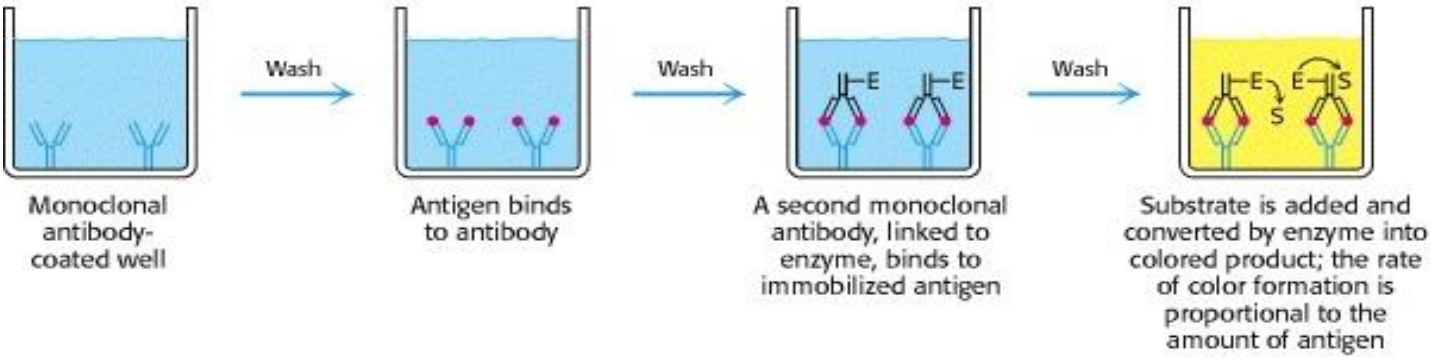
1- Increased sensitivity, since more than one labeled antibody is bound per primary antibody.
2- A wide variety of labeled secondary antibodies are available commercially.
3- Maximum immunoreactivity of the primary antibody is retained because it is not labeled.
4- Versatile because many primary antibodies can be made in one species and the same labeled secondary antibody can be used for detection.
5- Flexibility, since different primary detection antibodies can be used with a single labeled secondary antibody.
6- Cost savings, since fewer labeled antibodies are required.
7- Different visualization markers can be used with the same primary antibody.

Disadvantages

1- Cross-reactivity might occur with the secondary antibody, resulting in nonspecific signal.
2- An extra incubation step is required in the procedure.

2. Sandwich ELISA

Antigen can be detected by sandwich ELISA. In this technique, antibody is coated on the microtiter well. A sample containing antigen is added to the well and allowed to react with the antibody attached to the well, forming antigen-antibody complex. After the well is washed, a second enzyme-linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen. Then after unbound secondary antibody is removed by washing. Finally substrate is added to the plate which is hydrolyzed by enzyme to form colored products.



Procedure of sandwich ELISA

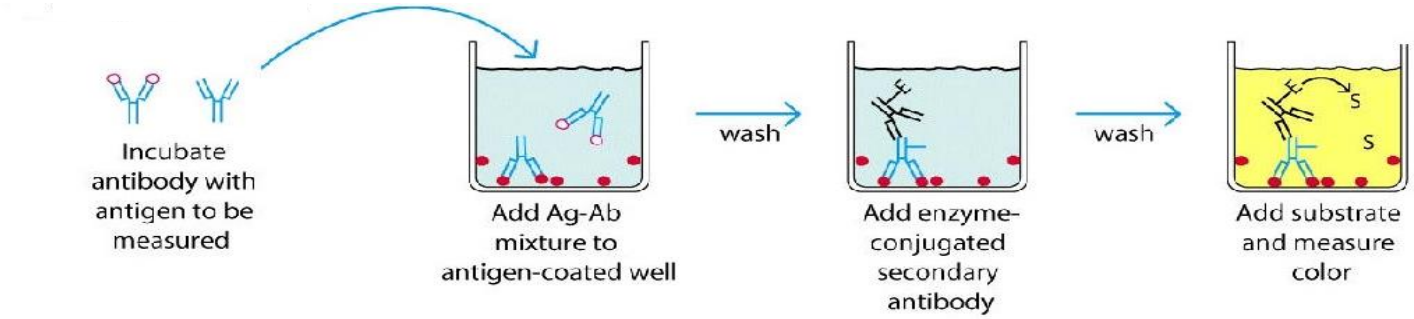
1. Prepare a surface to which a known quantity of antibody is bound.
2. Add the antigen-containing sample to the plate and incubate the plate at 37°c.
3. Wash the plate, so that unbound antigen is removed.
4. Add the enzyme-linked antibodies which are also specific to the antigen and then incubate at 37°c.
5. Wash the plate, so that unbound enzyme-linked antibodies are removed.
6. Add substrate which is converted by the enzyme to produce a colored product.
7. Reaction of a substrate with the enzyme to produce a colored product.

Advantages

1- High specificity, since two antibodies are used the antigen is specifically captured and detected.
2- Suitable for complex samples, since the antigen does not require purification prior to measurement.
3- Flexibility and sensitivity, since both direct and indirect detection methods can be used.

3. Competitive ELISA

This test is used to measure the concentration of an antigen in a sample.
In this test, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody mixture is then added to the microtitre well which is coated with antigen. The more the antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well. After the well is washed, enzyme conjugated secondary antibody specific for isotype of the primary antibody is added to determine the amount of primary antibody bound to the well. The higher the concentration of antigen in the sample, the lower the absorbance.



Procedure

1. Antibody is incubated with sample containing antigen.

2. Antigen-antibody complex are added to the microtitre well which are pre-coated with the antigen.

3. Wash the plate to remove unbound antibody.

4. Enzyme linked secondary antibody which is specific to the primary antibody is added.

5. Wash the plate, so that unbound enzyme-linked antibodies are removed.

6. Add substrate which is converted by the enzyme into a fluorescent signal.

Advantages

1- High specificity, since two antibodies are used.

2- High sensitivity, since both direct and indirect detection methods can be used.

3- Suitable for complex samples, since the antigen does not require purification prior to measurement.

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.