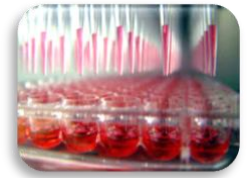


Lab. 8

Enzyme - Linked Immunosorbent Assay

Also known as

ELISA



The ELISA is a rapid test used for detecting and quantifying antibodies or antigens against viruses, bacteria and other materials. This method can be used to detect many infectious agents affecting poultry and livestock. In ELISA technology, the solid phase consists of a 96-well polystyrene plate, although other materials can be used. The function of the solid phase is to immobilize either antigens or antibodies in the sample, as they bind to the solid phase. After incubation, the plates are washed to remove any unbound material. In some assays the conjugate is then added to the plate and allowed to incubate. The conjugate consists of either an antigen or antibody that has been labeled with an enzyme. Depending upon the assay format, the immunologically reactive portion of the conjugate binds with either the solid phase or the sample. The enzyme portion of the conjugate enables detection. The plates are washed again and an enzyme substrate (hydrogen peroxide and a chromogen) is added and allowed to incubate. Color develops in the presence of bound enzyme and the optical density is read with an ELISA plate reader.

NOTE: The steps and reagents used can vary in an ELISA assay. It is best to reference the test insert for specific information on the technology for the assay being worked with.

ELISA Types

ELISAs are divided into several types.

Indirect ELISA: In the indirect format, the sample antibody is sandwiched between the antigen coated on the plate and an enzyme-labeled, anti-species globulin conjugate. The addition of an enzyme substrate-chromogen reagent causes color to develop. This color is directly proportional to the amount of bound sample antibody. The more antibody present in the sample, the stronger the color development in the test wells. This format is suitable for determining total antibody level in samples.

Competitive ELISA: In this format, the specific sample antibodies compete with, or block, the enzyme-labeled, specific antibody in the conjugate. The addition of an enzyme substrate-chromogen reagent causes color to develop. This color is inversely proportional to the amount of bound sample antibody. The more antibodies present in the sample, the less color development in the test wells.

Antigen-Capture (Direct) ELISA: In the antigen-capture format, the antigen in the sample is sandwiched between antibodies coated on the plate and an enzyme-labeled conjugate. The antibody conjugate can be either monoclonal or polyclonal. The addition of an enzyme substrate-chromogen reagent causes color to develop. This color is directly proportional to the amount of the target antigen present in the sample.

ELISA kit components

Coated Plates

The 96-well plates are made of polystyrene and coated with either inactivated antigen or antibody. This coating is the binding site for the antibodies or antigens in the sample. Unbound antibodies or antigens in the sample are washed away after incubation.

Sample Diluent

Most assays require a specific dilution of the sample. Samples are added to the sample diluent and mixed prior to putting them onto the coated plates.

Controls

The positive control is a solution that contains antibody or antigen. The negative control is a solution without antibody or antigen. The controls help to normalize or standardize each plate. Controls are also used to validate the assay and to calculate sample results. In most tests, the controls are prediluted and ready to use. Be sure to follow the instructions in the package insert.

Conjugate

ELISA conjugates are enzyme-labeled antibodies or antigens that react specifically to plate-bound sample analytes. Unbound conjugate is washed away after incubation and before the addition of substrate. The optical density of the colorimetric substrate is directly proportional to the quantity of bound enzyme present.

Substrate

For peroxidase conjugates, the substrate is a mixture of hydrogen peroxide and a chromogen that reacts with the enzyme portion of the conjugate to produce color.




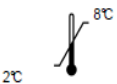









Wash Concentrate

The wash concentrate is a buffered solution containing detergent used to wash away unbound materials from the plates.

Stop Solution

The stop solution stops the enzyme-substrate reaction and, thereby, the color development.

Important signs that you might see on ELISA kit

												
Lot-Number	European conformity	For <i>in-vitro</i> diagnostic use	Temperature Limit	Use before	Catalogue Number	Consult instructions for use	Refer to accompanying documents	Do not use when package is damaged	Do not Re-use	Sufficient for <n> tests	Manufactured by	Biohazard