ELISA: Enzyme-linked immunosorbent assay

ELISA (Enzyme-linked immunosorbent assay) is one of immunoassay method using antibodies to capture an antigen and an enzyme labeled antibody to estimate the amount of antigen.

What is immunoassay?

The term “immunoassay” is a combined term of “immuno” (= immunological, practically immunochemical antigen-antibody-reaction) and “assay” (= determination of the purity of asubstance or the amount of any particular constituent of a mixture according to DorlandMedical Dictionary). So, immunoassay means a method to measure any particular substancein a mixture using its specific-binding antibody. One of the merits of immunoassay is that we can measure a substance that is present in a mixture of various contaminants, for example, one constituent of blood without any purification process.

What is ELISA?

The name ELISA derived from enzyme-linked immunosorbent assay. This assay method utilizes enzyme as a labelling material, and solidified antibody to capture target antigen. In ELISA a plate with 96 wells (well-plate) is used, and wells are coated with antibodies. These antibodies are called “capture antibodies”, the role of which is to capture the target antigen molecules in the sample. Coating is carried out by adsorption on the surface of bottom area. The well-plate is made of polystyrene which is modified for highly efficient adsorption. Because the concentration of antibody is related to efficiency of capturing antigen for excellent sensitivity, antibody preparation is used as IgG fraction, or monospecific antibody fraction obtained by an affinity chromatography rather than crude gamma-globulin fraction prepared by ammonium sulfate fractionation.

A basic procedure of ELISA

1) Standard solutions or assay samples are added to the antibody-coated wells, and incubated for several hours so as to the antigen molecules are captured by “capture antibody”.

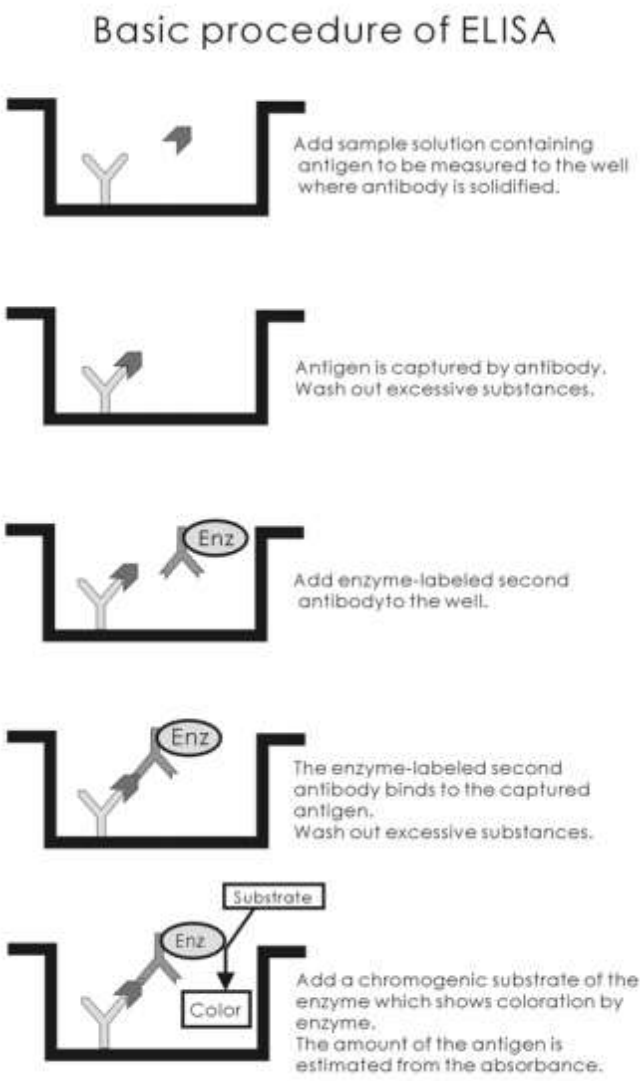
2) After this binding reaction, the reaction mixture is discarded, and wells are washed to remove excessive materials.

3) The second antibody which recognizes another epitope in antigen is added. This second antibody has been labeled with an enzyme such as horseradish peroxidase (HRP).

4) The enzyme-labeled second antibody will bind to the antigen which is bound to the capture 10 antibody on the bottom area of wells. This means that the enzyme (HRP) is also fixed on the bottom of wells. The amount of the antigen captured is proportional to fixed enzyme.

5) Enzyme activity is measured by adding a chromogenic substrate of this enzyme. In the case of HRP, tetramethylbenzidine (TMB) is often used. After incubation for some period, the chromogenic substrate is changed to a colored product. The reaction is stopped by adding a reaction stopper, e.g. diluted sulfuric acid, and absorbance is measured using a plate reader.

6) The standard curve is prepared from the concentration of standard solutions and their absorbance. And the sample assay values are obtained from the absorbance using the standard curve (calibration curve).



Suggested readings: **Engvall, E (1972-11-22). "Enzyme-linked immunosorbent assay, Elisa". The Journal of Immunology. 109 (1): 129–135. ISSN 0022-1767. PMID 4113792**