



Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Enzyme Linked Immunosorbent Assay (ELISA) Technique Guideline

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ARTICLE INFO

Keywords:

ELISA
Antigen
Antibody
Guideline

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All authors have reviewed and approved the final version of the manuscript.

<https://doi.org/10.32539/bsm.v5i5.228>

ABSTRACT

ELISA (Enzyme-linked immunosorbent assay) is a technique used to assess the quantification of peptide, protein, antibody and hormone levels, based on the principle of antigen-antibody binding. In the ELISA technique, antigen immobilization will be carried out on a solid surface, then bound with antibodies to form an antigen-antibody bond complex, where the antigen-antibody complex is bound to the enzyme. The detection signal in the form of a color change will be formed due to the reaction between the enzyme and the substrate.

1. Introduction

ELISA is generally carried out on a plate containing 96 wells, where the antibody binding process with protein will occur. This very simple process makes ELISA easy to do. Ease of ELISA for washing, making it easier to clean the following materials when tested. This will make testing with ELISA specific.

The ELISA examination procedure begins with the process of attaching (coating) the antigen and / or antibody to the surface of the well on the plate. Furthermore, the blocking step is carried out (blocking) antigen and antibody bonds at the unspecific-site with a blocking agent. After incubation and washing, the plates were incubated with enzyme-bound antibodies. Next, washing the plates was carried out and continued with the addition of the substrate so that the color

change would be produced and the OD (optical density) value was read with an ELISA reader.

The washing stage is an important one to remove antibodies that are not bound to antigens. In addition, make sure that no washing liquid is left on the plate, because it is feared that it will affect the next stage of the inspection.

Terminology

- ELISA plate: a container for antigen-antibody collection, which generally contains 96 test wells.
- Antigen: A protein to be assessed for levels, derived from the sample to be tested. If we are going to assess IL-12 levels by using ELISA from serum

samples, then the IL-12 contained in our serum is termed an antigen.

- **Antibody:** A protein that binds to an antigen. If we are going to assess IL-12 levels using ELISA from a serum sample, then an antibody will be added, then the antibody is a protein that will bind to IL-12, or known as anti-IL-12.
- **Standard:** A protein that will be graded, which has known levels. If we are going to assess IL-12 levels using ELISA from serum samples, the standard is the IL-12 protein that has known levels, for example the standard IL-12 level is 100 pg / mL. Generally, this standard will be made into several concentrations, so that you will get a graph depicting the standard VS the OD value of the standard. The graph will be used to calculate the levels in the sample.
- **OD (Optical Density) value:** a value that describes the intensity of the color change on ELISA
- **ELISA reader:** Tool used to get the OD value from an ELISA examination.

ELISA type

There are several types of ELISA, namely: direct, indirect, sandwich or competitive. In the immobilization stage the antigen can be attached directly to the plate or indirectly to the plate, by binding with antibodies that have been attached to the bottom of the plate. Furthermore, the antigen is detected directly with the primary antibody bound to the enzyme or the antigen is detected indirectly with the secondary antibody that is bound to the primary antibody and the enzyme. Enzymes that are generally bound to antibodies include: alkaline phosphatase (AP) or horseradish peroxidase (HRP).

Direct ELISA

In this type, the antigen is attached to the bottom of the *plate*, then the antigen will be detected through the antibody bound to the enzyme.

Advantages:

- Examination with this method is faster

- Cross-reaction with secondary antibodies can be eliminated

Disadvantages:

- The resulting signal amplification is weak
- Lack of flexibility in selecting enzyme labeled primary antibodies
- There may be reactions between primary antibodies and enzymes bound to these primary antibodies.

Indirect ELISA

In this method, the antigen is attached to the base of the *plate*, then, the primary antibody which is not labeled with the enzyme is inserted. Next, put back the enzyme labeled secondary antibody, which will bind to the primary antibody.

Advantages:

- The sensitivity of the test is increased with the use of primary and secondary antibodies.

Disadvantages:

- Cross-reactions can occur with secondary antibodies which will result in a non-specific signal.
- Longer incubation time is required
- The cost required is greater than the direct method

ELISA sandwich

In this method, the antibody is first attached to the base of the *plate*. Next, the test sample (antigen) is inserted into the well on the *plate*, then a secondary antibody bound to the enzyme is inserted into the well on the *plate*.

Advantages:

- Has high specificity
- Suitable for use with less pure samples

Disadvantages:

- The cost is quite large because it uses two antibodies

Competitive ELISA

Competitive ELISA is an ELISA examination

method where there is a competitive reaction between the sample antigen and antigen bond that is attached to the bottom of the *plate* well with the primary antibody. In this method, the non-sample antigen is attached to the bottom of the plate. Next, the sample antigen and primary antibody are inserted into the well. Then put secondary antibodies that are bound to the enzyme in the wells on the plate.

ELISA data interpretation

The final result of the ELISA examination will be obtained as the OD value (optical density). However, the OD value is not the level of the ELISA examination, so it is still necessary to process the OD value data. The first thing to do is to make a standard OD value curve VS standard content so that the line equation will be obtained from the curve. The equation obtained will be used to convert the OD value of the sample into a grade value.

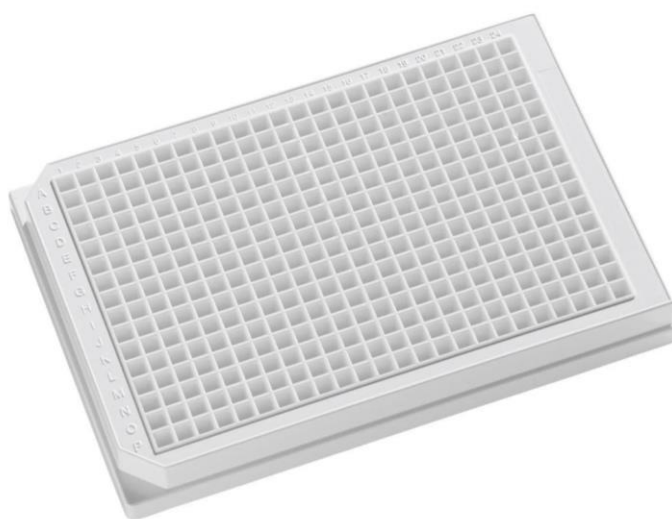


Figure 1. ELISA plate.



Figure 2. ELISA antibody.



Figure 3. Standard ELISA.

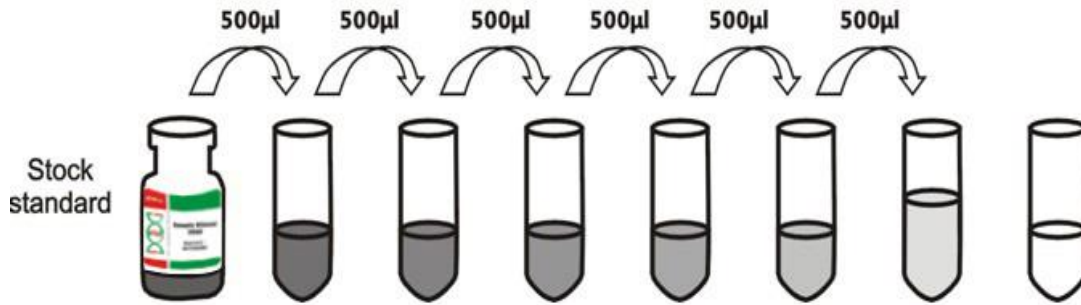


Figure 4. Making serial concentration standard.



Figure 5. Changes in color intensity on ELISA plate.



Figure 6. ELISA reader

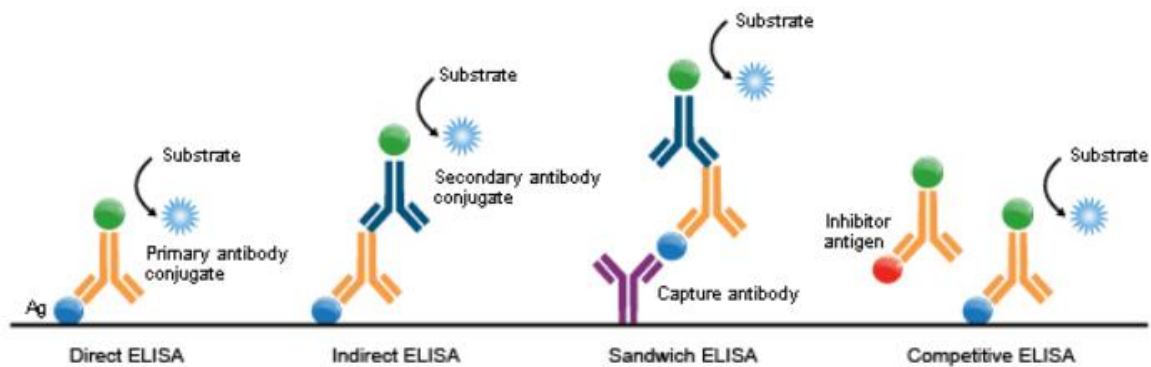


Figure 7. ELISA types.

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