# Experiment (5): Enzyme-Linked Immunosorbent Assay (ELISA)

## Aim:

- To understand the principle of different types of ELISA.
- To detect the insulin level in Rat plasma sample using Ultra Sensitive Mouse Insulin ELISA Kit

## Introduction:

Immunoassay is a test that uses the highly specific and selective antigen-antibody reactions forming antibody and antigen complexes [immuno-complexes] as a means of generating measurable results. Antigen (Ag) is a substance that when introduced into the body stimulates the production of an *antibody*. Antigens include toxins, bacteria, foreign blood cells, and the cells of transplanted organs. Antibodies (Ab) are large Y-shaped glycoproteins. They are produced by the immune system in response to foreign objects (*antigen*) to identify and neutralize them. Each antibody recognizes a specific antigen (not normally found in the body).[1,2,3]

PAUSE AND THINK ➔ What called the specific part of the antigen that is recognized by the antibody?

ELISA (enzyme-linked immunosorbent assay) is a biochemical plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. ELISAs are typically performed in 96-well (or 384-well) polystyrene microtiter plates, where antibody or antigen of interest is immobilized. [4] In qualitative ELISA format, the results provide a positive or negative result for a sample whereas in quantitative ELISA, the optical density or florescent units of the sample is interpolated into a standard curve (obtained from serial dilutions of a standard). [5]

ELISA can be used in the field of medicine, food industry and in toxicology labs to evaluate the presence of a specific Ag or Ab in a sample. Different applications include Screening donated blood for evidence of viral contamination and measuring hormones level.[6]

PAUSE AND THINK ➔ Can we use ELISA to detect autoimmune diseases? How?

## Basic Principle:

The basic principle of ELISA is, to detect a specific antibody- antigen reaction by assessing the conjugated enzyme activity which can convert a colourless substrate to a measurable coloured product, indicating the presence of the antibody - antigen [Ab-Ag] binding. The detection enzyme can be linked directly to the primary antibody or introduced through a secondary antibody that recognizes the primary antibody. The most crucial element of the detection strategy is a highly specific antibody-antigen interaction. [4]

## ELISA format:

ELISAs can be performed with a number of modifications to the basic procedure:

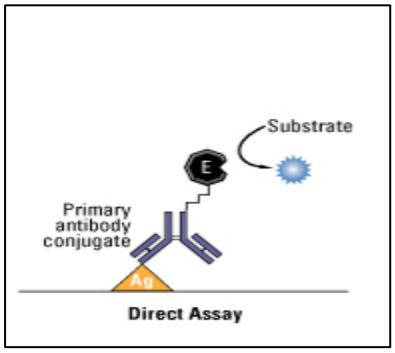
(**1**) Direct ELISA. (**2**) Indirect ELISA. (**3**) Sandwich ELISA. (**4**) Competitive ELISA.

### 1. Direct ELISA:

This type considered the simplest type of ELISA. It is used to detect the presence and the concentration of specific antigen in the sample.

- **Principle:**

Immobilization of the antigen of interest can be accomplished by direct adsorption or fixation to the assay plate. The antigen is then directly detected by an antibody conjugated with an enzyme. By adding, the enzyme's substrate, the enzyme will convert colourless substrate to coloured product. The colour produced is proportional the amount of the antigen of interest.



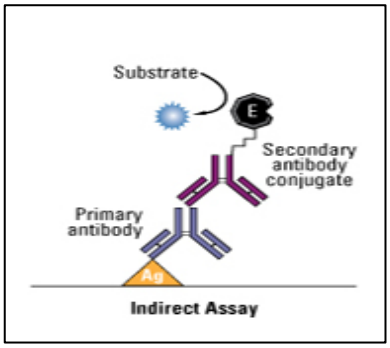

PAUSE AND THINK ➔ Why this format called "direct ELISA" ?

### 2. Indirect ELISA:

Is used to detect the presence and the concentration of specific antigen or antibody.

- **Principle:**

This method differs than direct ELISA, in that one more labelled secondary antibody is added in the reaction. The antigen is first captured by primary antibody (which can be the interest), then a secondary enzyme conjugated antibody is added which recognizes the primary antibody. The color or the signal produced as a result of addition of substrate is proportional to antigens/antibodies in the sample.



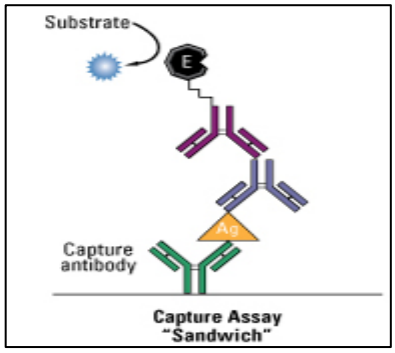

PAUSE AND THINK ➔ Why this format called "Indirect ELISA" ?

### 3. Sandwich ELISA:

The most powerful ELISA assay format is the sandwich assay. In sandwich ELISA the antigen in indirectly captured by antibody immobilized in the microtiter plate. This type of capture assay is called a “sandwich” assay because the analyte to be measured is bound between two primary antibodies – the capture antibody and the detection antibody. It is used to detect the presence and the concentration of specific antigen in the sample. The sandwich format is used because it is sensitive and robust. (4)

➢ **Principle:**

The sandwich ELISA quantify/detect antigens between two layers of antibodies (i.e. capture and detection antibody just like a sandwich). The antigen to be measured must contain at least two antigenic epitopes since at least two antibodies bind to the antigen. The colour or the signal produced as a result of addition of substrate is proportional to antigen concentration.



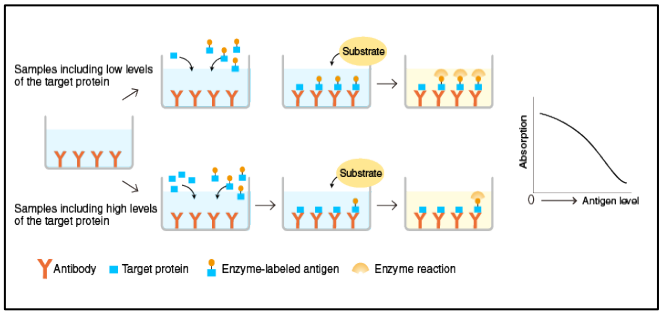

PAUSE AND THINK ➔ What is the main difference between direct/indirect ELISA and sandwich ELISA?

## 4. Competitive ELISA:

Is a strategy that is commonly used when the antigen is small and has only one epitope, or antibody binding site. It measures the amount of antigen in a sample. One variation of this method consists of labelling purified antigen instead of the antibody.

➢ **Principle:**

In this type of ELISA, another version of your antigen of interest is labelled instead of the antibody. Unlabelled antigen "your interest" and the "labelled antigen" compete for binding to the capture antibody (antibody that fixed in the plate). The colour or the signal produced as a result of addition of substrate is inversely proportional to antigens of interest in the sample. (4)




PAUSE AND THINK ➔ What are the differences between this format and standard previous format?

## Material & Protocol

According to Ultra Sensitive Mouse Insulin ELISA Kit (Cat# 90080)

### Supporting materials:

- Overview of ELISA and supporting videos: https://www.thermofisher.com/sa/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-elisa.html

### References:

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2. https://courses.lumenlearning.com/boundless-microbiology/chapter/antibodies/
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