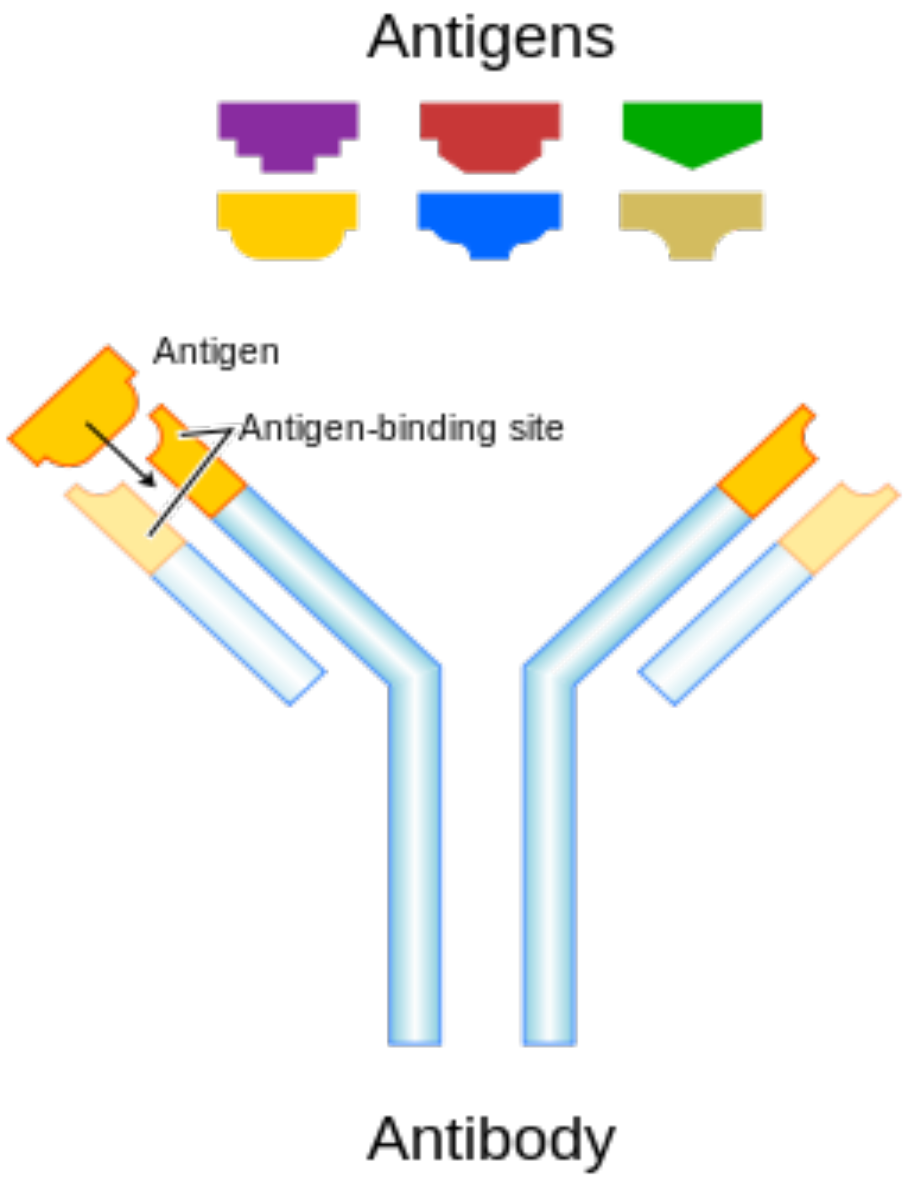
ENZYME-LINKED IMMUNOSORBENT ASSAY [ELISA]

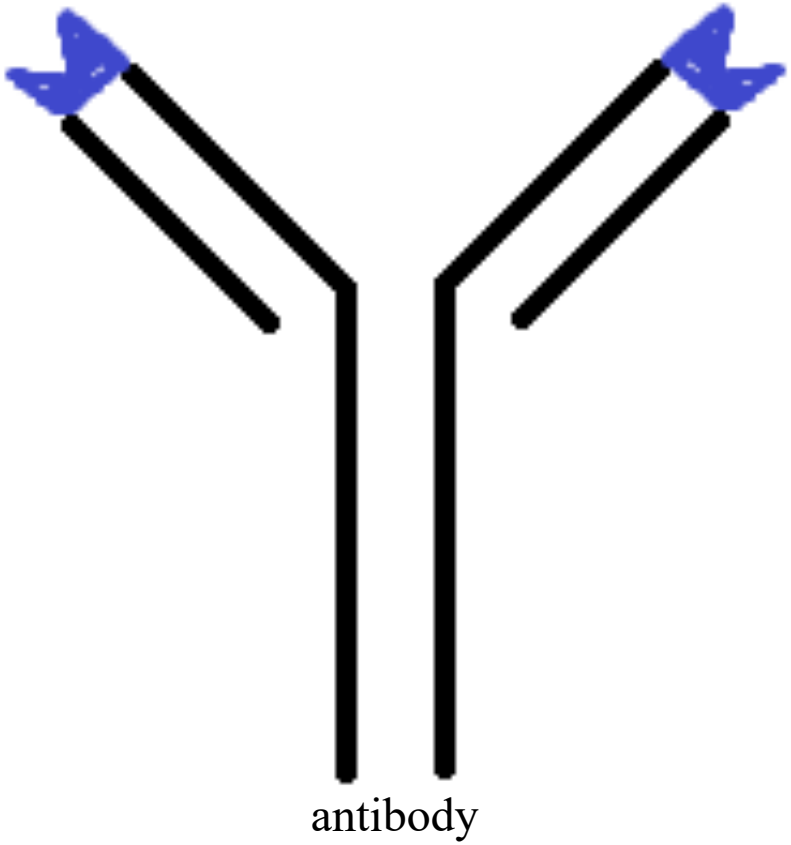
BCH462- Practical

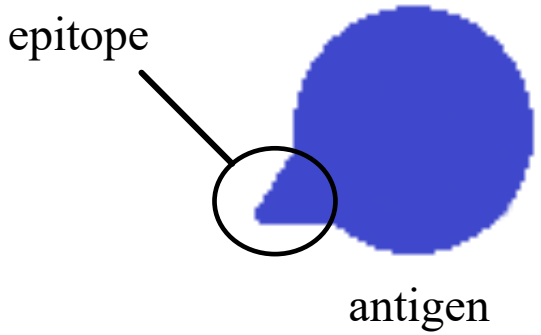
Immunoassay:

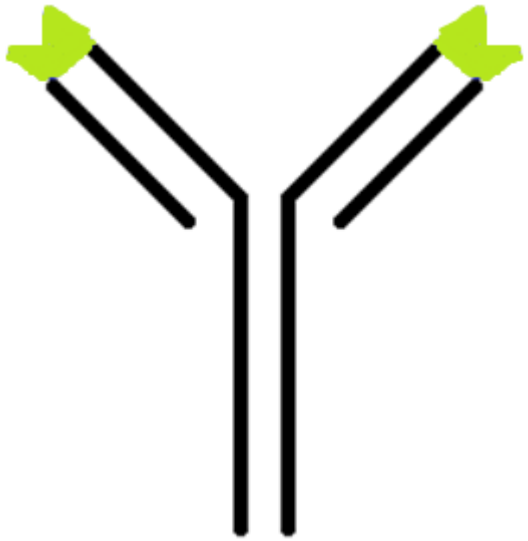
- What is antigen (Ag) ?
- What is antibody (Ab) ?
- Immunoassay ?
- Specificity ?



Each antibody recognize specific antigen

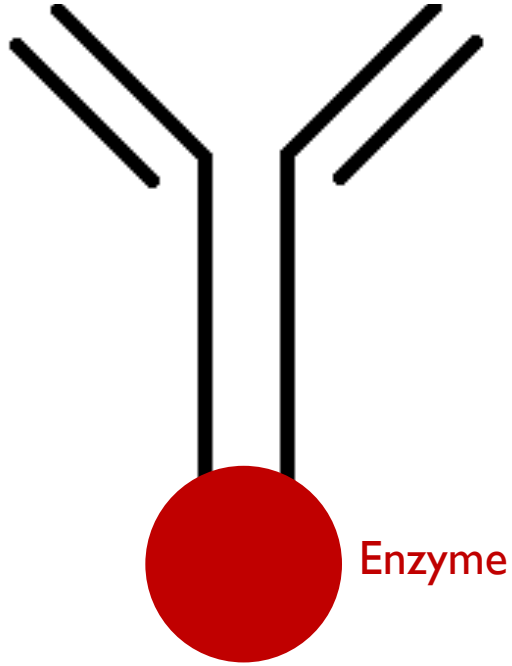

antibody


epitope
antigen



Primary antibody

"antibody specified to **specific antigen**"



Secondary antibody

"antibody specified to Primary antibody"

ELISA:

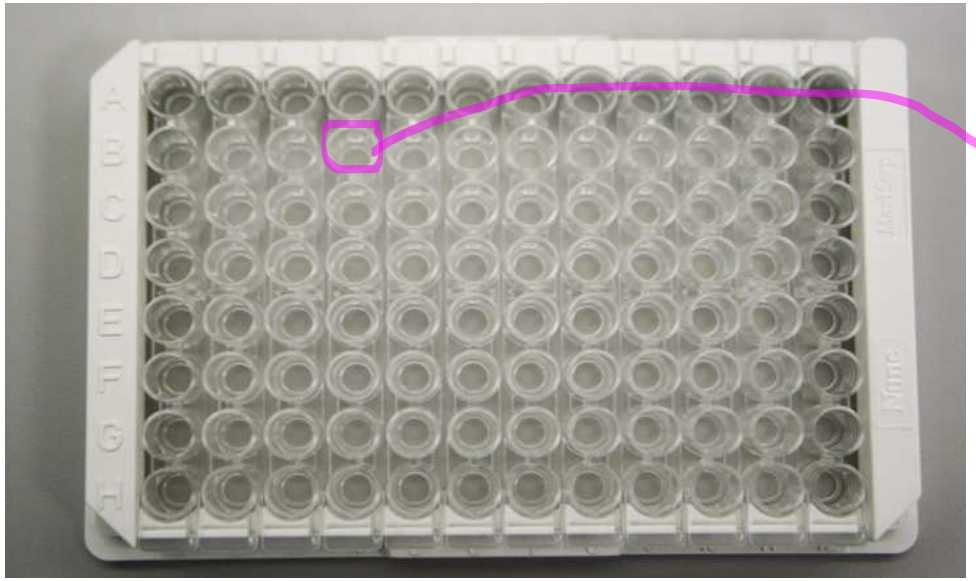
- Enzyme-linked immunosorbent assay.
- Is a biochemical plate-based assay technique designed for **detecting and quantifying** substances such as peptides, proteins, antibodies and hormones.

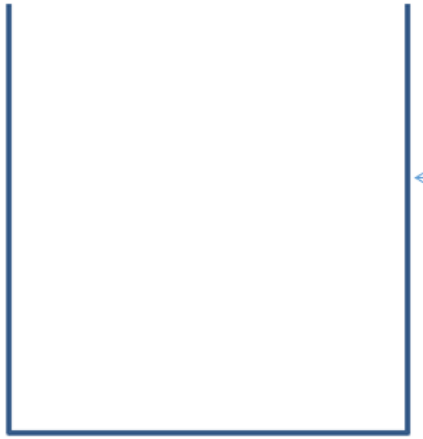
➔ In qualitative ELISA: (+ OR -)

➔ In quantitative ELISA: The optical density or florescent units of the sample is interpolated into a standard curve.

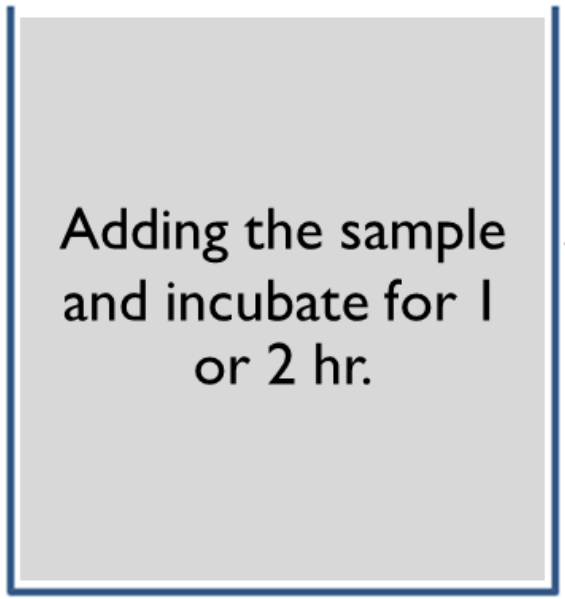
- Application ?
- Autoimmune disease?

96-well (or 384-well) polystyrene microtiter plates





microtitre plate
solid support used to immobilized antigen or antibody of interest.



microtitre plate
solid support used to immobilized antigen or antibody of interest.

Basic Principle:

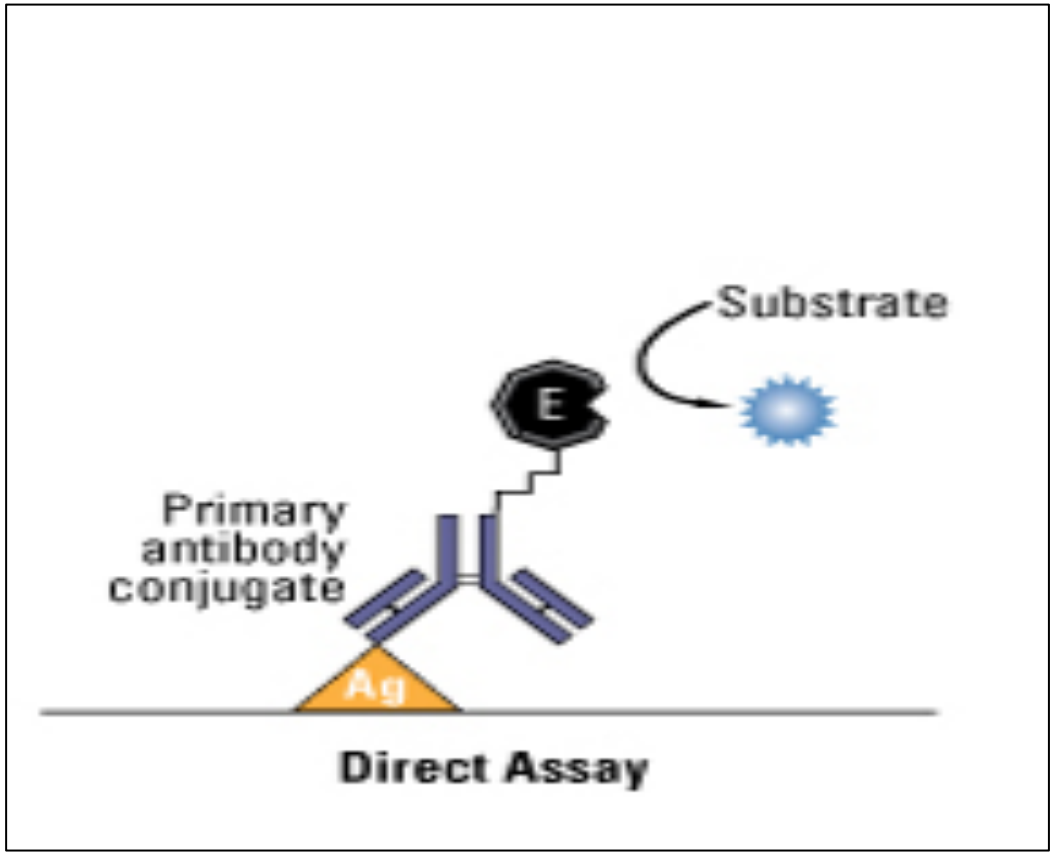
- To detect a specific antibody- antigen reaction by assessing the ***conjugated enzyme activity.***
- The enzyme convert a colourless substrate to a measurable coloured product, indicating the presence of the antibody - antigen [Ab-Ag] binding.
- The detection enzyme can be linked **directly to the 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.

ELISA Format:

- Direct ELISA.
- Indirect ELISA.
- Sandwich ELISA.
- Competitive ELISA.

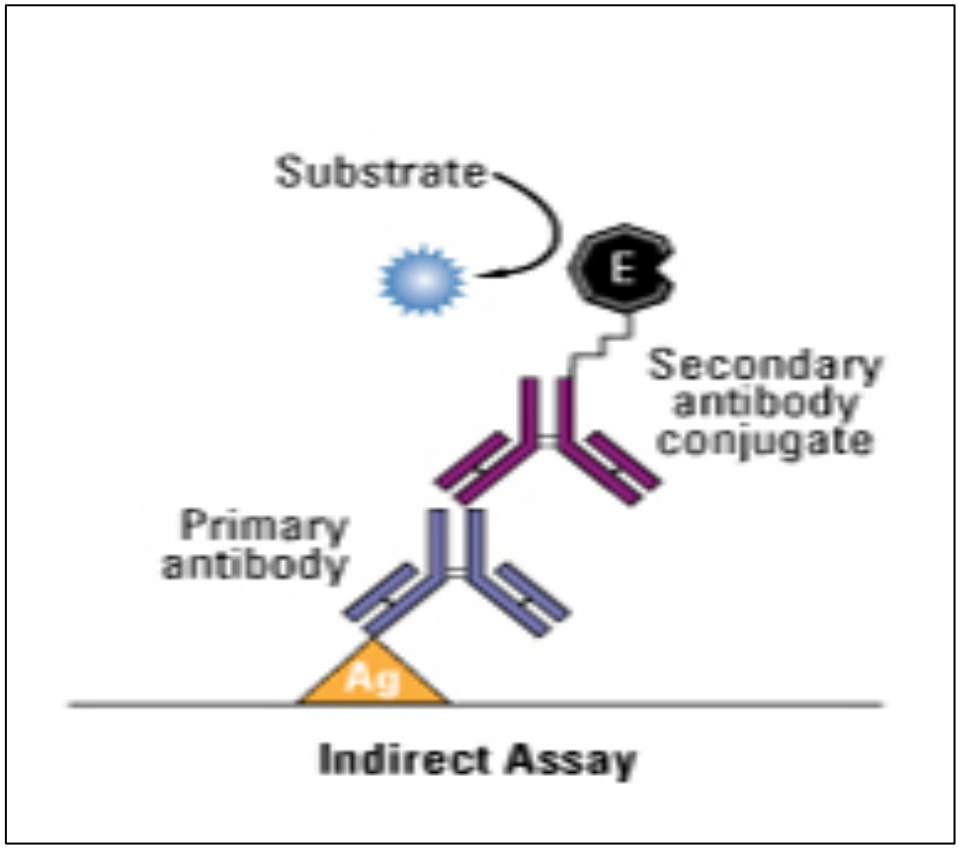
Direct ELISA:

- This type considered the simplest type of ELISA.
- It is used to detect the presence and the concentration of specific antigen in the sample.
- Why this format called "direct ELISA" ?



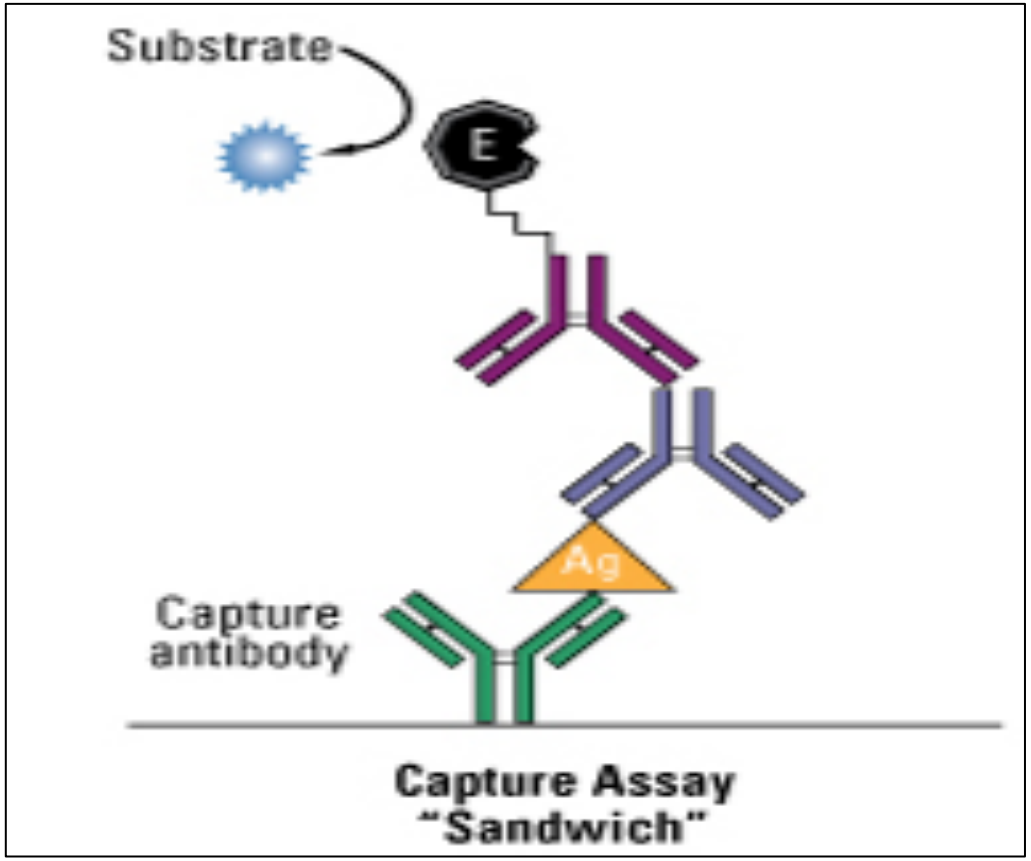
Indirect ELISA:

- Is used to detect the presence and the concentration of specific antigen or antibody.
- Why this format called “Indirect ELISA" ?



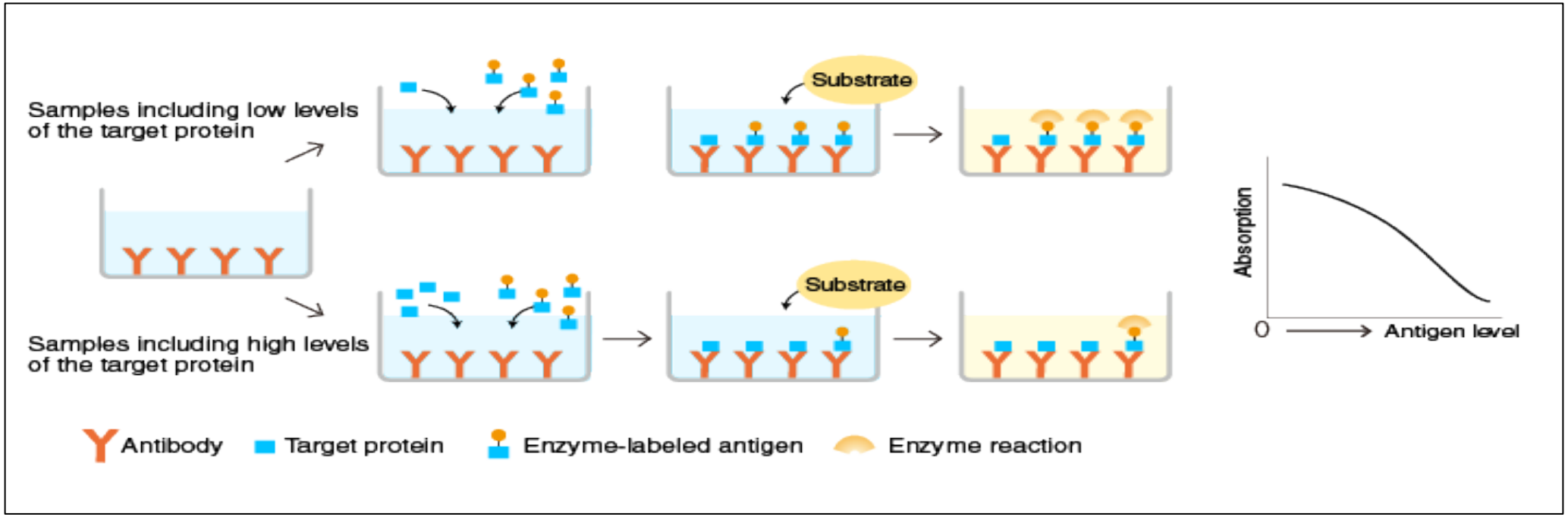
Sandwich ELISA:

- The most powerful ELISA assay format is the sandwich assay.
- 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.



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.**



PRACTICAL PART

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

Workflow

add 95µl of sample diluent with (2, 5, 7.5 and 10µl of sample) to each well as duplicate.

↓

Incubate for 2 hours at 4 °C

↓

Wash plate with 300µl washing buffer (repeat three times)

↓

Add 100µl of conjugate solution to each well

↓

Incubate 30 minutes at room temperature.

↓

Wash plate with 300µl washing buffer (repeat three times)

↓

Add 100µl of substrate solution to each well

↓

Incubate 40 minutes at room temperature in the dark

↓

Add 100µl of stop solution to each well

↓

Measure OD at 450 nm whithin 30 minutes

Results

- Table (1): The absorbance and concertation values of insulin standard solutions

Absorbance at 450nm		Insulin concentration (ng/ml)
1.508	1.499	6.4
0.856	0.956	3.2
0.419	0.458	1.6
0.243	0.209	0.8
0.116	0.147	0.4
0.065	0.070	0.2
0.032	0.038	0.1

- Take the average of standard solution absorbance, and plot standard curve.

Results

- Table (2): The absorbance and concertation values of insulin in plasma sample

	Absorbance at 450nm	Insulin concentration (ng/ml)
10 ul		
5 ul		
2.5 ul		
1 ul		

- Take the average of sample absorbance , and find the concentration using the curve.