BCH 462- Biotechnology & Genetic engineering [Practical]

Lab (5) Enzyme-linked Immunosorbent Assay [ELISA]

Immunoassay

- **Immunoassay:**

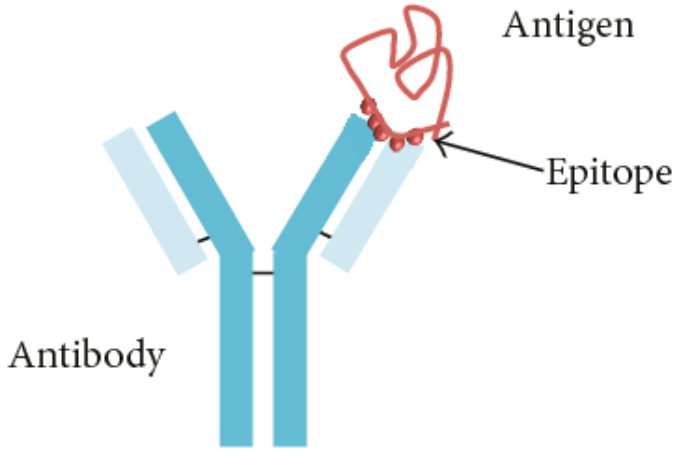
A test that uses highly specific and selective antigen-antibody reactions forming **antibody-antigen complexes [immuno-complexes]** as a means of generating measurable results.

- **Antigens [Ag]:**

A substance that when introduced into the body stimulates the production of an antibody.

- **Antibody [Ab]:**

- Antibodies (Ab) are large Y-shaped glycoproteins, 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).



Remember !!
Protein of interest = Antigen or Antibody

Immunoassay

Primary Antibody "antibody specified to specific antigen"

Antigen

Secondary Antibody "antibody specified to Primary antibody"

ELISA

ELISA (Enzyme-Linked ImmunoSorbent Assay) is a biochemical plate-based assay technique designed for detecting and quantifying substances, such as:

I. **Proteins (peptides, hormones) "antigens in general".**
II. **Antibodies.**

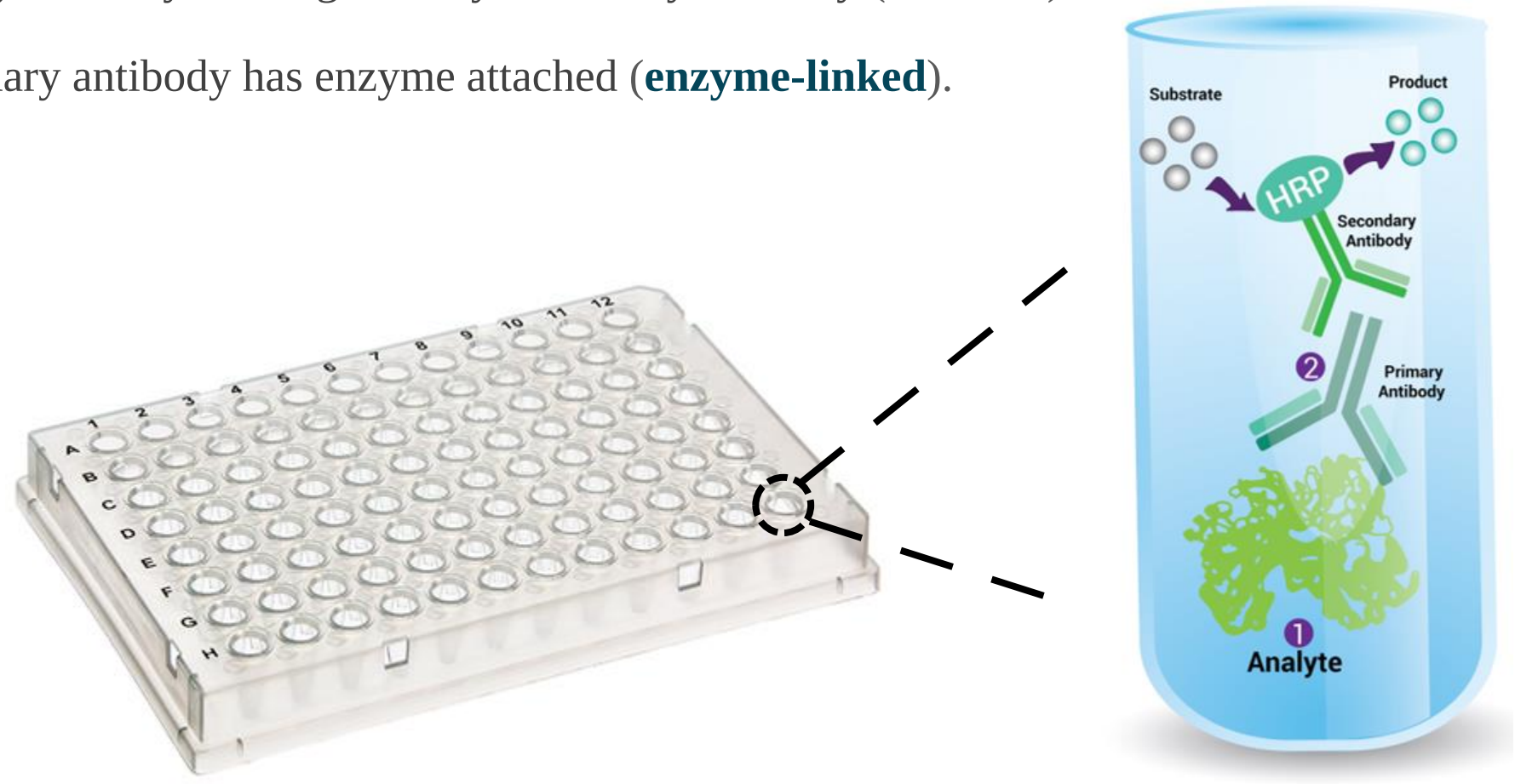
- ELISAs are typically performed in 96-well (or 384-well) polystyrene microtiter plates (solid phase), where antibody or antigen of interest is immobilized.

Propose of ELISA:

- To determine the presence and the concentration of a particular Ag or Ab in a sample, thus it can be run in a qualitative and quantitative format.

Enzyme Linked Immunosorbent Assay (ELISA)

1. Antigen of interest is absorbed on to plastic surface (**sorbent**).
2. Antigen is recognized by primary antibody (**immuno**).
3. Primary antibody is recognized by secondary antibody (**immuno**)
4. Secondary antibody has enzyme attached (**enzyme-linked**).



ELISA

1. In qualitative ELISA, determines antigen or antibody is **present or absent**
2. In quantitative ELISA, determines the quantity (concentration) of the antibody/antigen in a sample through **measuring absorbance** of the sample.
    - Requires standard curve (obtained from serial dilutions of a standard).

- **Applications:**

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.

They can be used for:

1. Can be used to diagnose disease such as **AIDS**.
2. Measuring hormones level (pregnancy testing **hCG**).

Pause and Think Can we use ELISA to detect autoimmune diseases? How?

ELISA Principle

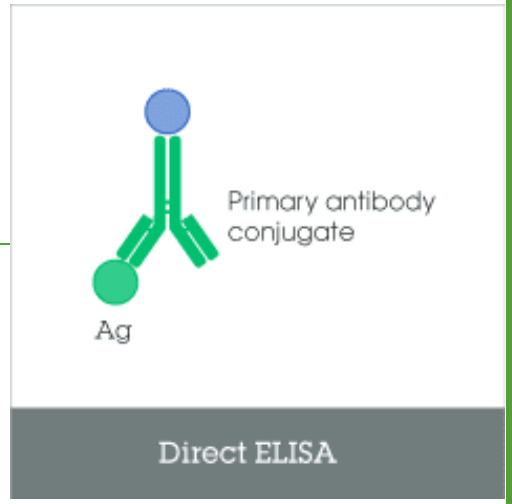
- To detect a specific antibody-antigen reaction by assessing the ***conjugated enzyme activity.***
- The enzyme convert a **colorless substrate** to a measurable colored product indicating the presence of the **antibody - antigen [Ab-Ag] binding**.
- The detection enzyme can be linked **directly to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

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



- Direct ELISA is considered to be the simplest type of ELISA.
- It is used to detect the presence and the concentration of specific **antigen** in the sample.

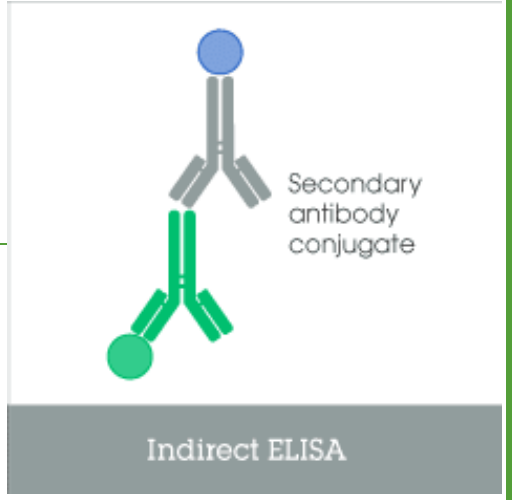
Principle

- The antigen "of interest" is adsorbed or fixed "immobilized" to a microtiter plate.
- An "enzyme is linked to an antibody" applied to the antigen, the enzyme-antibody, will bound to antigen of interest.
- By adding, the enzyme's substrate, the enzyme will convert colorless substrate to colored product.
- The color produced is proportional the amount of the antigen of interest.

  Pause and Think Why this format called "direct ELISA" ?

- The name "direct ELISA" due to, that (the antibody linked to the enzyme) is **directly** bind to the protein of interest "antigen".

2. Indirect ELISA



- It is used to detect the presence and the concentration of specific **antigen or antibody**.

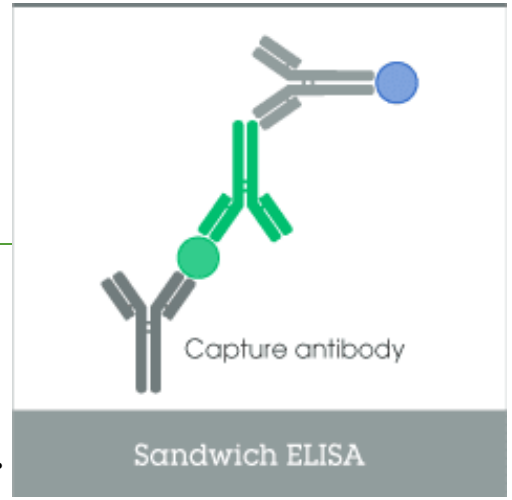
Principle

- 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" ?

- The name Indirect ELISA, is due to that secondary antibody bind **indirectly** to the antigen.

3. Sandwich ELISA

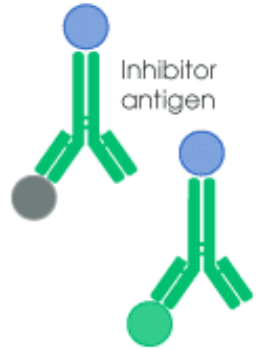


- The most powerful ELISA assay format is the sandwich assay. Why ?
- It is used to detect the presence and the concentration of specific **antigen** in the sample.

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 color or the signal produced as a result of addition of substrate is proportional to antigen concentration.

4. Competitive ELISA



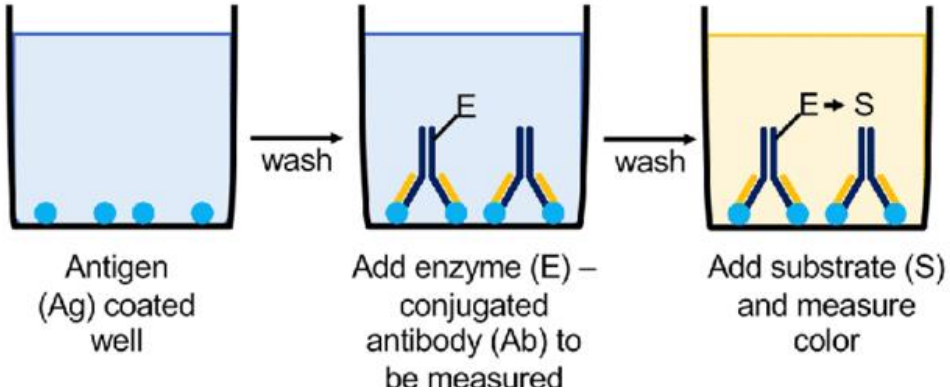
Competitive ELISA

- Also known as **inhibition 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 or antibody** in a sample.
- One variation of this method consists of labelling antigen instead of the antibody (reference antigen).

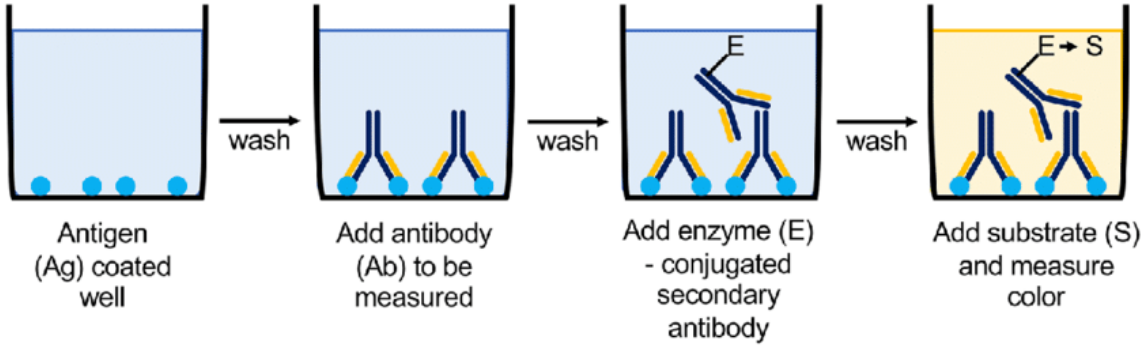
Principle

- In this type of ELISA, another version of your antigen of interest is labeled instead of the antibody.
- Unlabeled antigen "your interest" and the "labeled antigen" compete for binding to the capture antibody.
- The color or the signal produced as a result of addition of substrate is inversely proportional to antigens of interest in the sample.

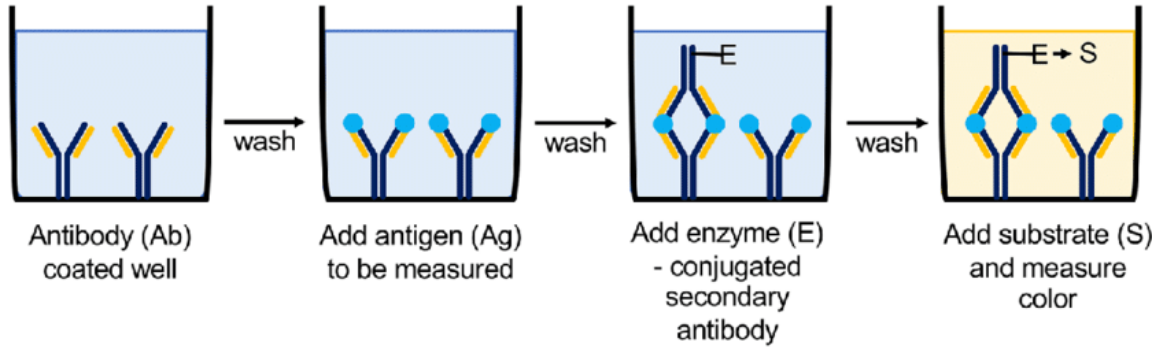
(a) Direct ELISA



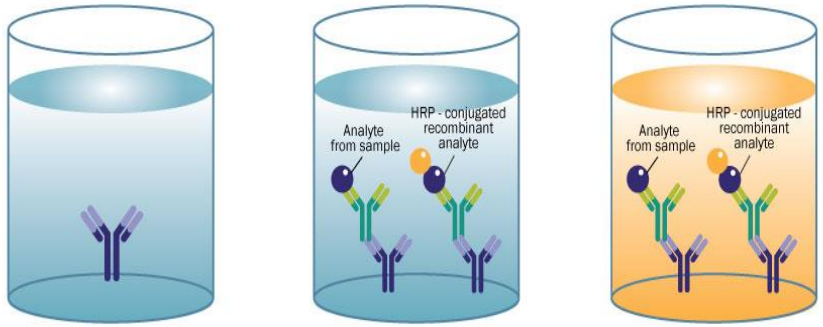
(b) Indirect ELISA



(c) Sandwich ELISA



(D) Competitive ELISA



Practical Part

Practical part

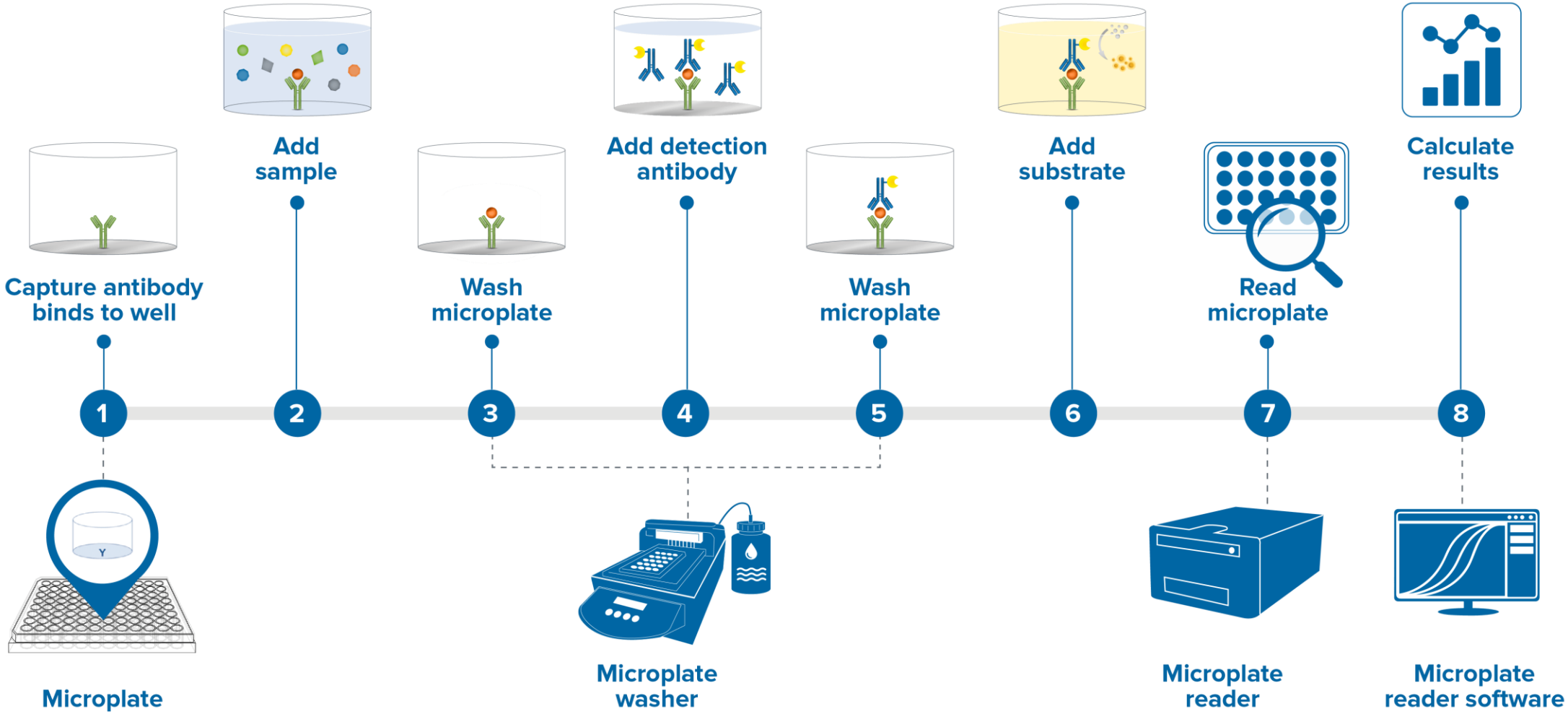
- **Aims:**
  - To detect the presence of the newcastle disease virus (NDV) antibody in *Gallus domesticus* using Chicken newcastle disease virus (NDV) antibody (IgG) ELISA Kit

- **Equipment**

96-Well Polystyrene Plates

ELISA (plate) reader

Multichannel pipette



Overview of ELISA technique

Workflow

Add 100 µl of of sample diluent to blank well and 100 µl of sample to sample well

Incubate for 30 minutes at 37°C

Wash plate with 250µl washing buffer (total of two washes)

Add 50µl of HRP-conjugate to each well (not to Blank!).

Incubate for 30 minutes at 37°C

Wash plate with 250µl washing buffer (total of two washes)

Add 100µl mixed substrate to each well

Incubate for 10 minutes at 37 °C. Protect from light.

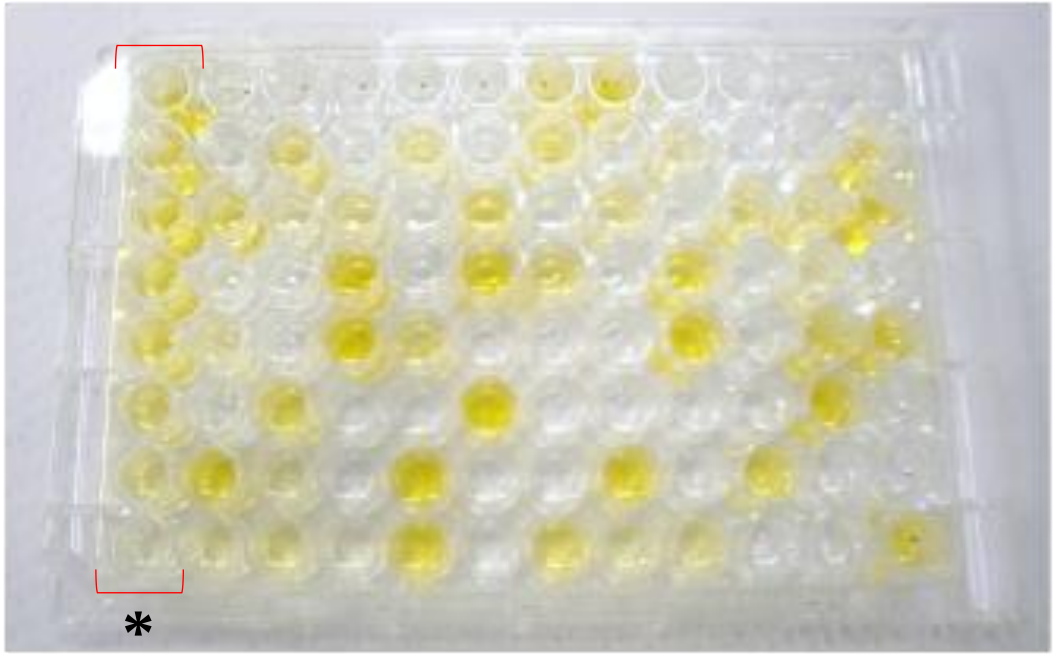
Add 50µl of Stop Solution to each well

Measure OD at 450 nm within 10 minutes

Results

Lane	Absorbance at 450nm
1	
2	
3	
4	
5	
6	
7	

ELISA plate at the end of the reaction



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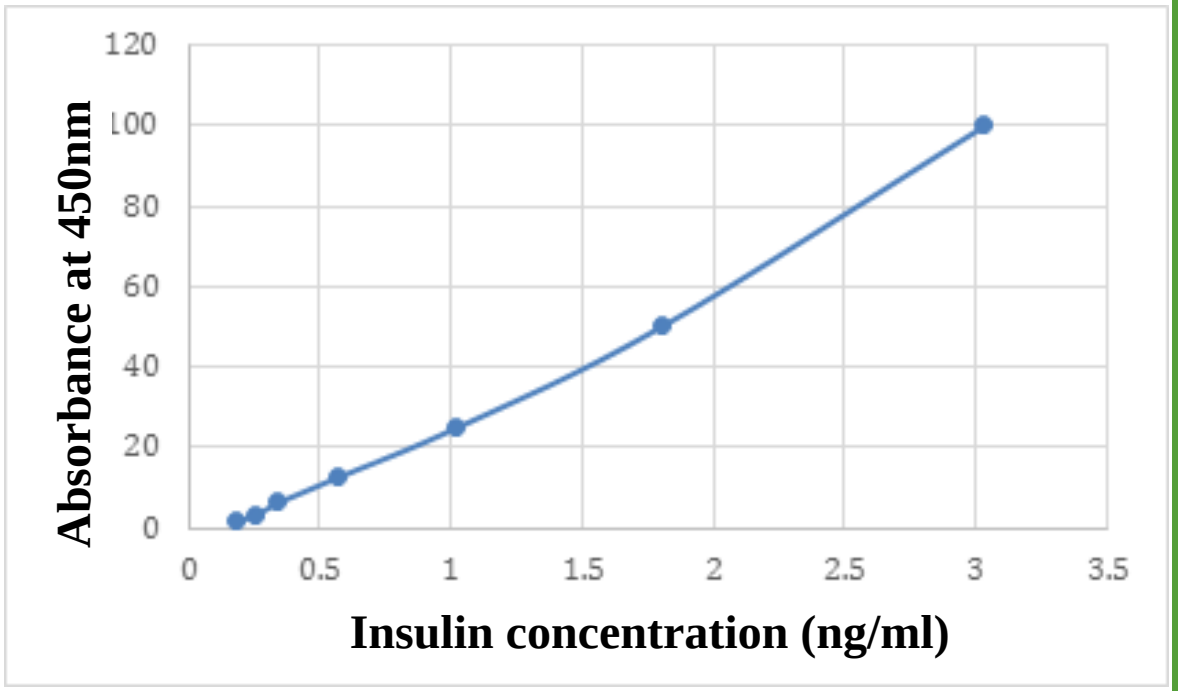
The yellow color indicates that the target protein is present. The higher degree of the color, the higher concentration of the target protein.

* **Lane 1** represents serial dilutions of the standard



ELISA standard curve

Homework

- Draw with labelling the four different ELISA formats.