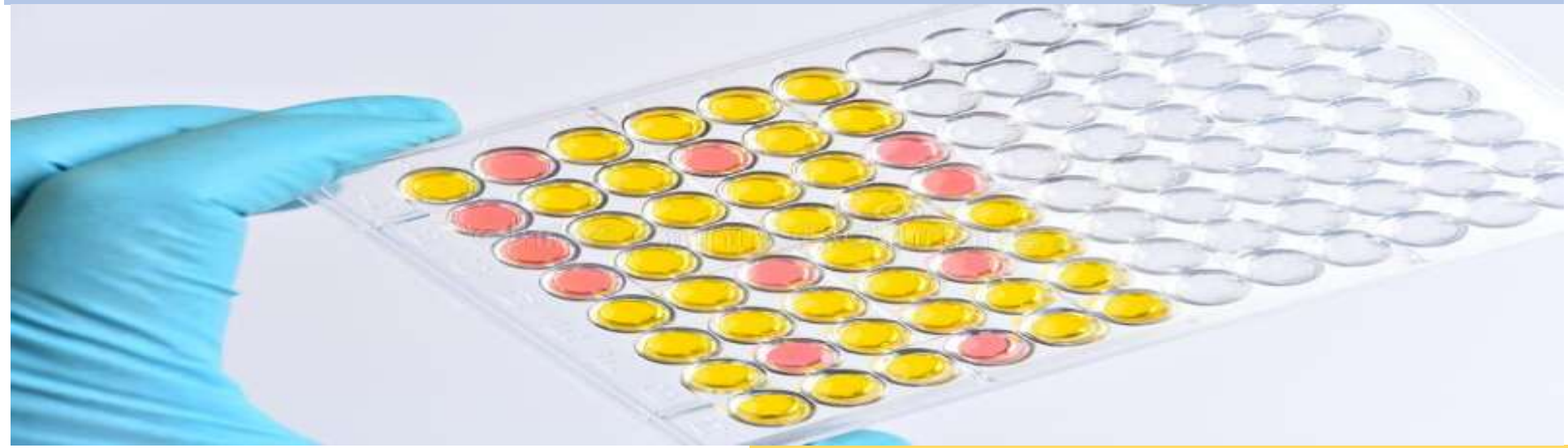
ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)



DR Kahkashan Perveen

BIOCHEMISTRY

LEARNING OBJECTIVES

At the end of this lecture 2nd year MBBS students will be able to:

- Define ELISA
- Describe the principle of ELISA
- Classify the types of ELISA tests.
- Enlist the applications and advantages of ELISA tests in medicine.

Immunoassays

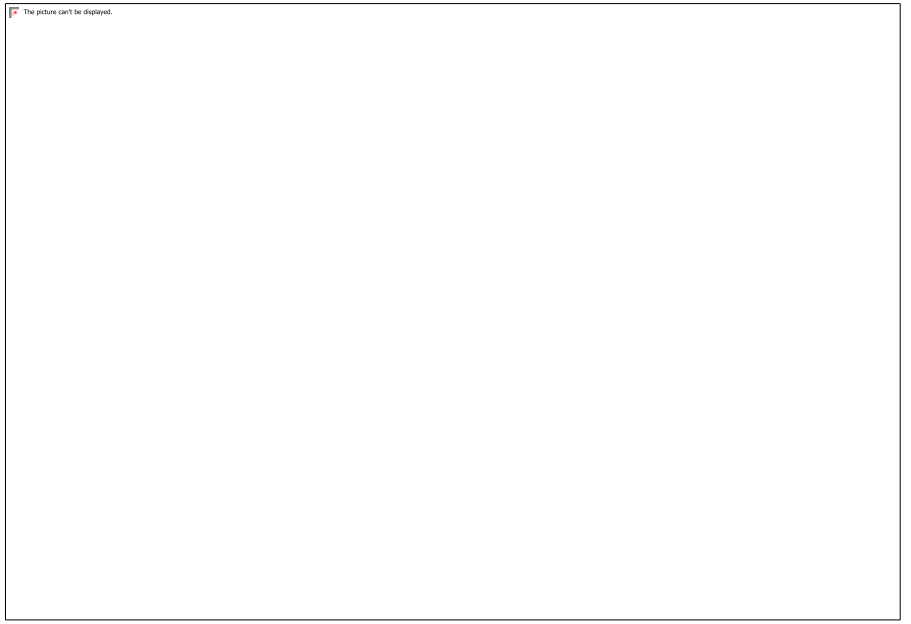
- Immunoassays are **bioanalytical methods** that use the specificity of an antigen-antibody reaction to detect and quantify target molecules in biological samples
- These methods are frequently used in:

➢ Clinical diagnostics

➢ Drug discovery

➢ Drug monitoring

➢ Food testing



Enzyme-linked immunosorbent assay (ELISA)

- Is an immunological assay
- Both Qualitative & Quantative process
- Commonly used to measure:

➢ **Antibodies**

➢ **Antigens**

➢ **Proteins**

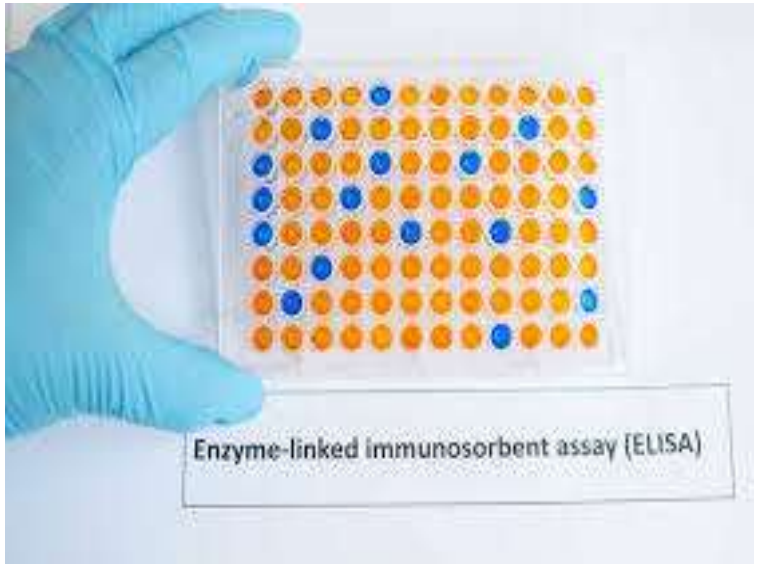
➢ **Peptides**

➢ **Glycoproteins**

➢ **Hormones** in biological samples

Enzyme-linked immunosorbent assay (ELISA)

- ELISA assays are generally carried out in 96 well plates (microtitre plate made up of Polystyrene PVC)
- Allowing multiple samples to be measured in a single experiment
- These plates need to be special absorbant plates (e.g. NUNC Immuno plates) to ensure the antibody or antigen sticks to the surface.
- Each ELISA measures a specific antigen/antibody
- Kits for a variety of antigens are widely available



Why called
Enzyme-linked immunosorbent assay

1) Antigen /Antibody of interest is absorbed onto plastic surface (sorbent)
2) Antigen is recognized by specific Antibody (Immuno)
3) This antibody is recognized by second Antibody (immune) which has enzyme attached (enzyme –linked)
4) Substrate reacts with enzyme to form product , usually colored

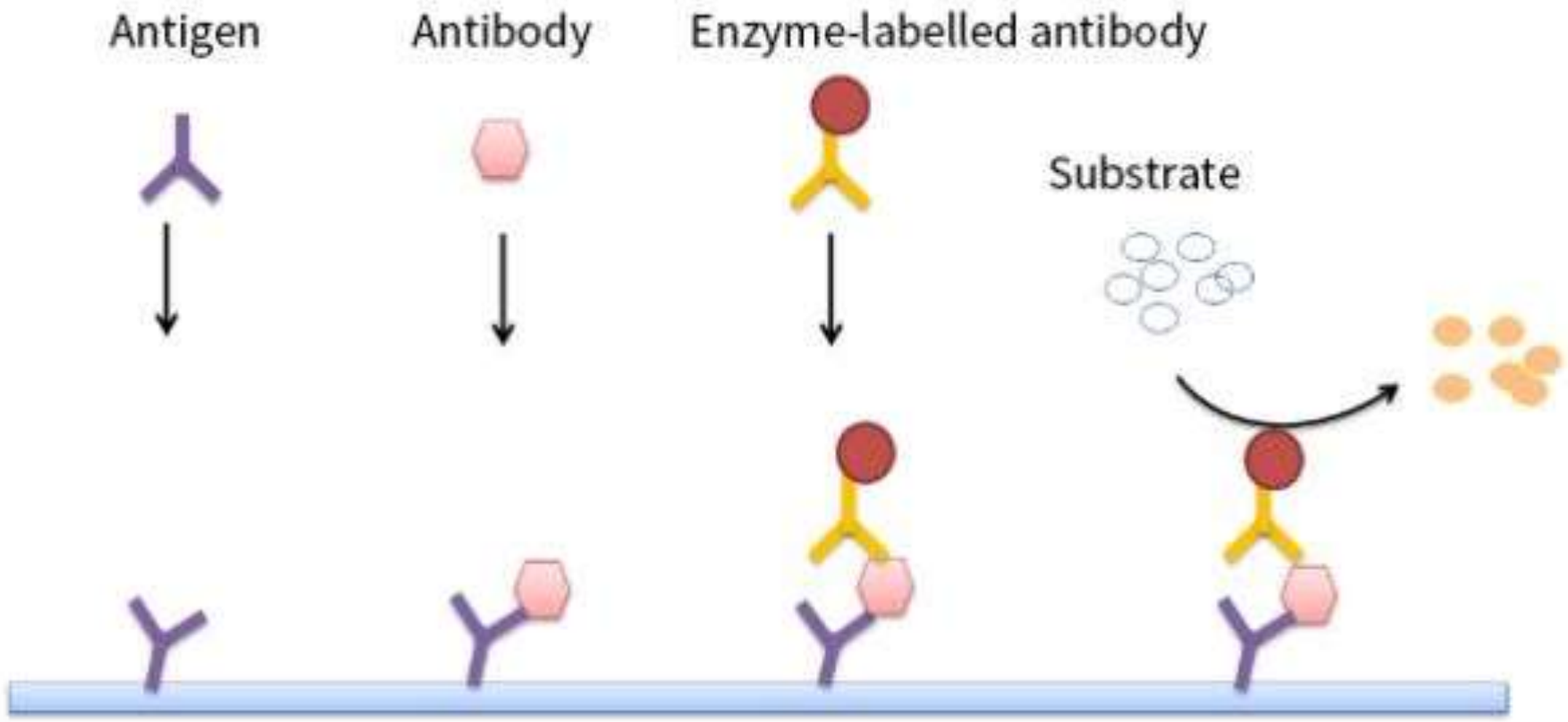
ELISA PRINCIPLE

The ELISA can be used to detect the presence of antigens that are recognized by an antibody or it can be used to test for antibodies that recognize an antigen.

A general ELISA is a five-step procedure

1) Coat the microtiter plate wells with antigen
2) Block all unbound sites to prevent false positive results
3) Add primary antibody (e.g. rabbit monoclonal antibody) to the wells
4) Add secondary antibody conjugated to an enzyme (e.g. anti-mouse IgG)
5) Reaction of a substrate with the enzyme to produce a colored product, thus indicating a positive reaction

Mechanism of ELISA



Mechanism of ELISA

- Performing an ELISA involves at least one antibody with specificity for a particular antigen.
- The sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene microtiter plate, either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a "sandwich" ELISA).

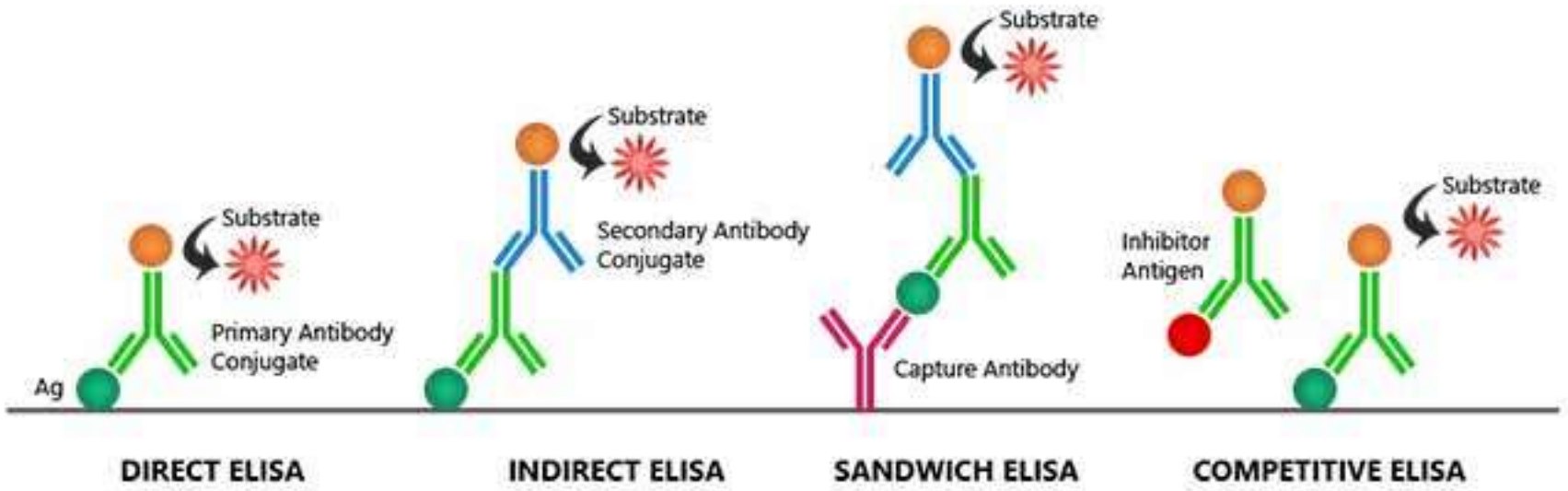
Mechanism of ELISA

- After the antigen is immobilized, the detection antibody is added, forming a complex with the antigen.
- The detection antibody can be covalently linked to an enzyme, or can itself be detected by a secondary antibody that is linked to an enzyme through bioconjugation.

Mechanism of ELISA

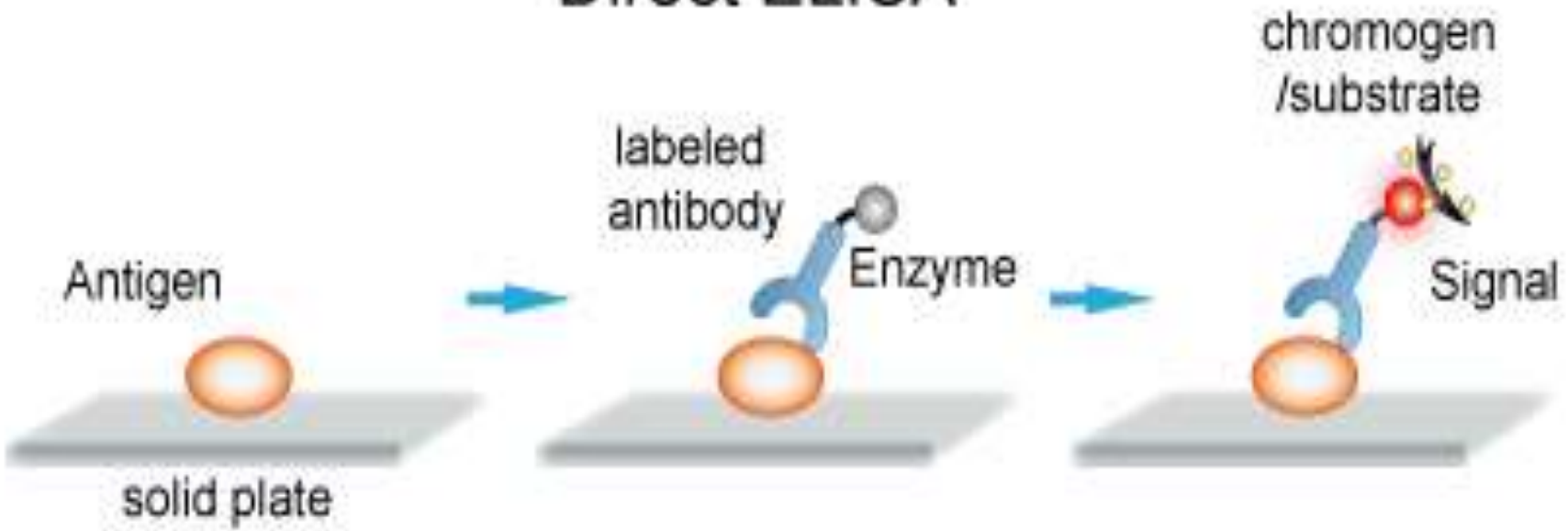
- Between each step, the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound.
- After the final wash step, the plate is developed by adding an enzymatic substrate to produce a visible signal
- which indicates the quantity of antigen in the sample.

Types of ELISA



For antigen	For antibody	For antigen	For small antigen

Direct ELISA

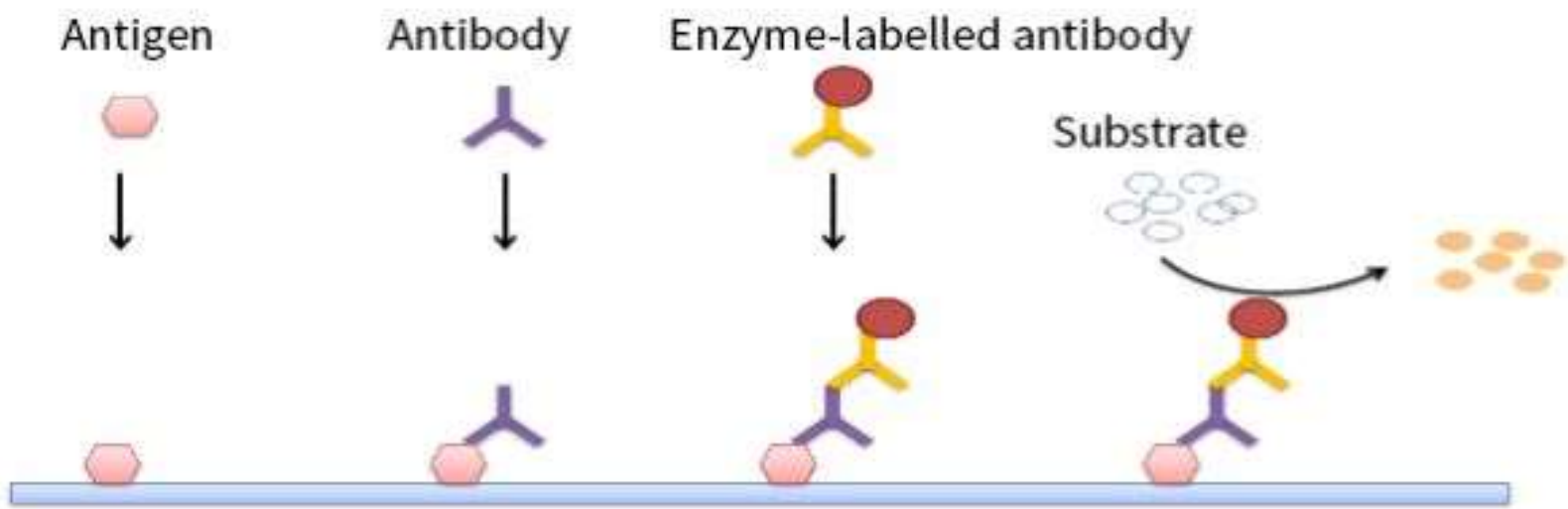


1. Antigen is coated onto wells by passive adsroption
2. Antibody conjugated with enzyme is added and incubated with antigen.and incubation.
3. Substrate / chromophore is added and colour develops.

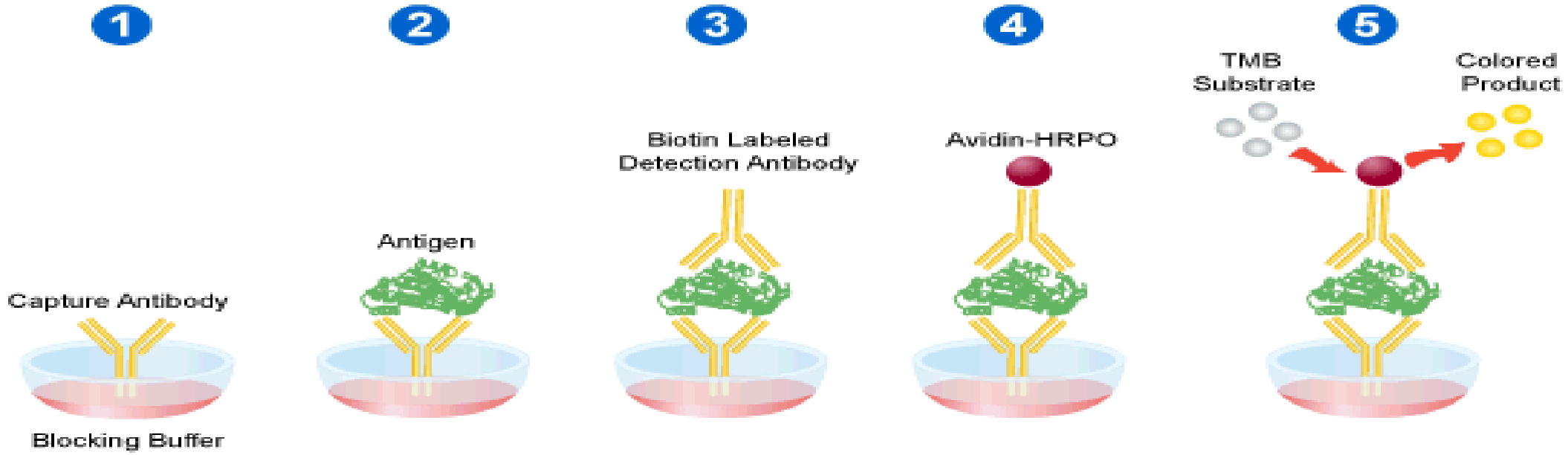
Indirect ELISA

Indirect ELISA is a two-step ELISA which involves two binding process of primary antibody and labeled secondary antibody. The primary antibody is incubated with the antigen followed by the incubation with the secondary antibody. However, this may lead to nonspecific signals because of cross-reaction that the secondary antibody may bring about.

1. Micro-well plates are incubated with antigens, washed up and blocked with BSA.
2. Samples with antibodies are added and washed.
3. Enzyme linked secondary antibody are added and washed.
4. A substrate is added, and enzymes on the antibody elicit a chromogenic or fluorescent signal.



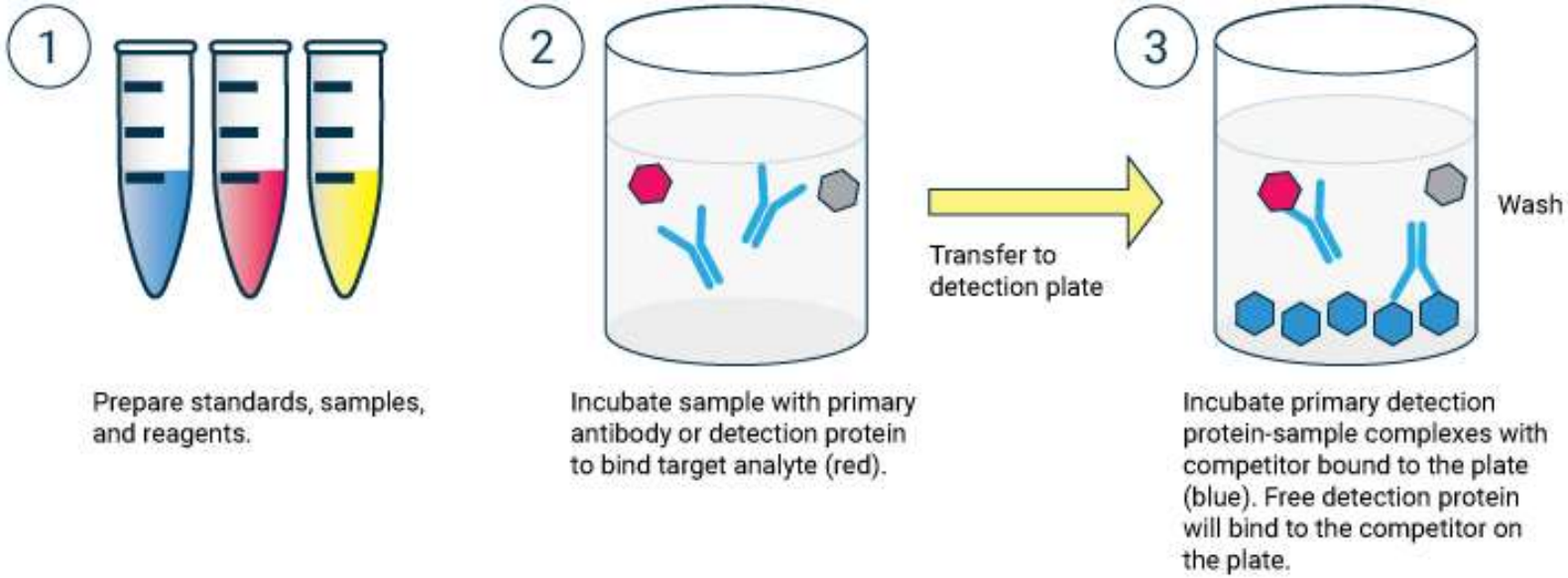
Sandwich ELISA



1. **a.)** Plate is coated with a suitable capture antibody. **b.)** Blocking buffer is added to block remaining protein-binding sites on plate.
2. Sample is added to plate and any antigen present is bound by the capture antibody.
3. A suitable biotin labeled detection antibody is added to the plate and also binds to any antigen present in well.
4. UltraAvidin™-HRPO *(Leinco Prod. No. A106)* is added and binds the biotin labeled detection antibody.
5. TMB substrate *(Leinco Prod. No. T118)* is added and converted by HRPO to a detectable form.

HPRO HORSE RADISH PER OXIDASE
TMB (TETRA METHYBENZIDINE)

Competitive ELISA



Advantages of ELISA

- More accurate.
- Highly sensitive, specific and compare favorably with other methods used to detect substances in the body, such as radioimmune assay (RIA) tests.
- Does not need radioisotopes (radioactive substances) or a costly radiation counter
- Useful biotechnical tool with many applications, either in scientific research or clinical diagnosis of diseases or conditions.

APPLICATIONS OF ELISA

- **Serum Antibody Concentrations**
- **Detecting potential food allergens**
- (milk, peanuts, walnuts, almonds and eggs)
- **Disease outbreaks- tracking the spread**
- **of disease**
- e.g. HIV, bird flu, common, colds, cholera,
- STD etc
- **Detections of antigens**
- e.g. pregnancy hormones, drug allergen,
- GMO, mad cow disease
- **Detection of antibodies in blood sample for**
- **past exposure to disease**
- e.g. Lyme Disease, trichinosis, HIV, bird flu


THANK YOU
FOR YOUR ATTENTION