ELISA

Enzyme Linked Immunosorbent Assay

What is an ELISA?

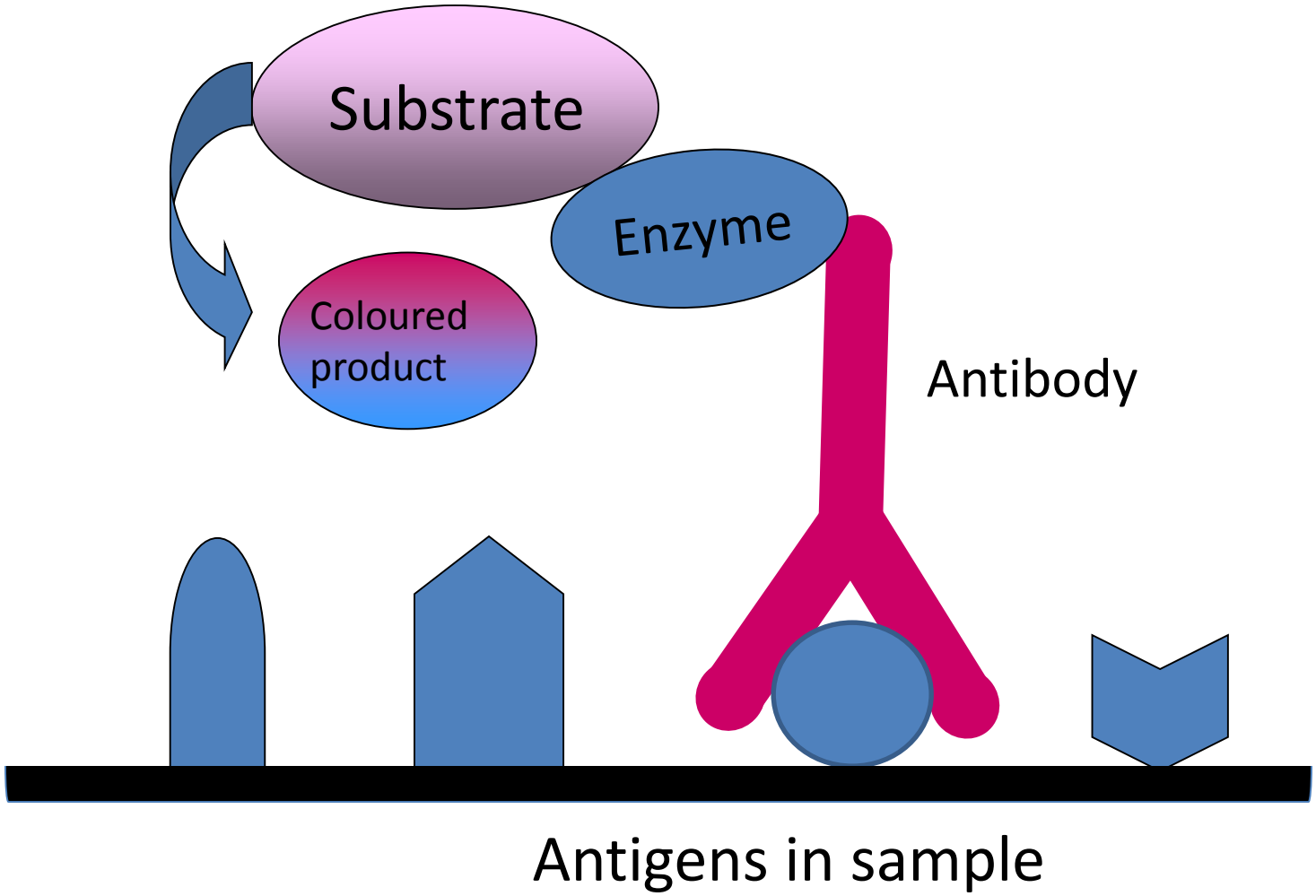
- **Immunological technique used to detect specific molecules (e.g. proteins & carbohydrates) in samples whether are antigen or antibodies**
- **Quantitative and very sensitive**

Types of ELISA

- Direct ELISA
- Indirect ELISA
- Sandwich ELISA

- All carried out in a 96 well micro titre plate

Direct ELISA

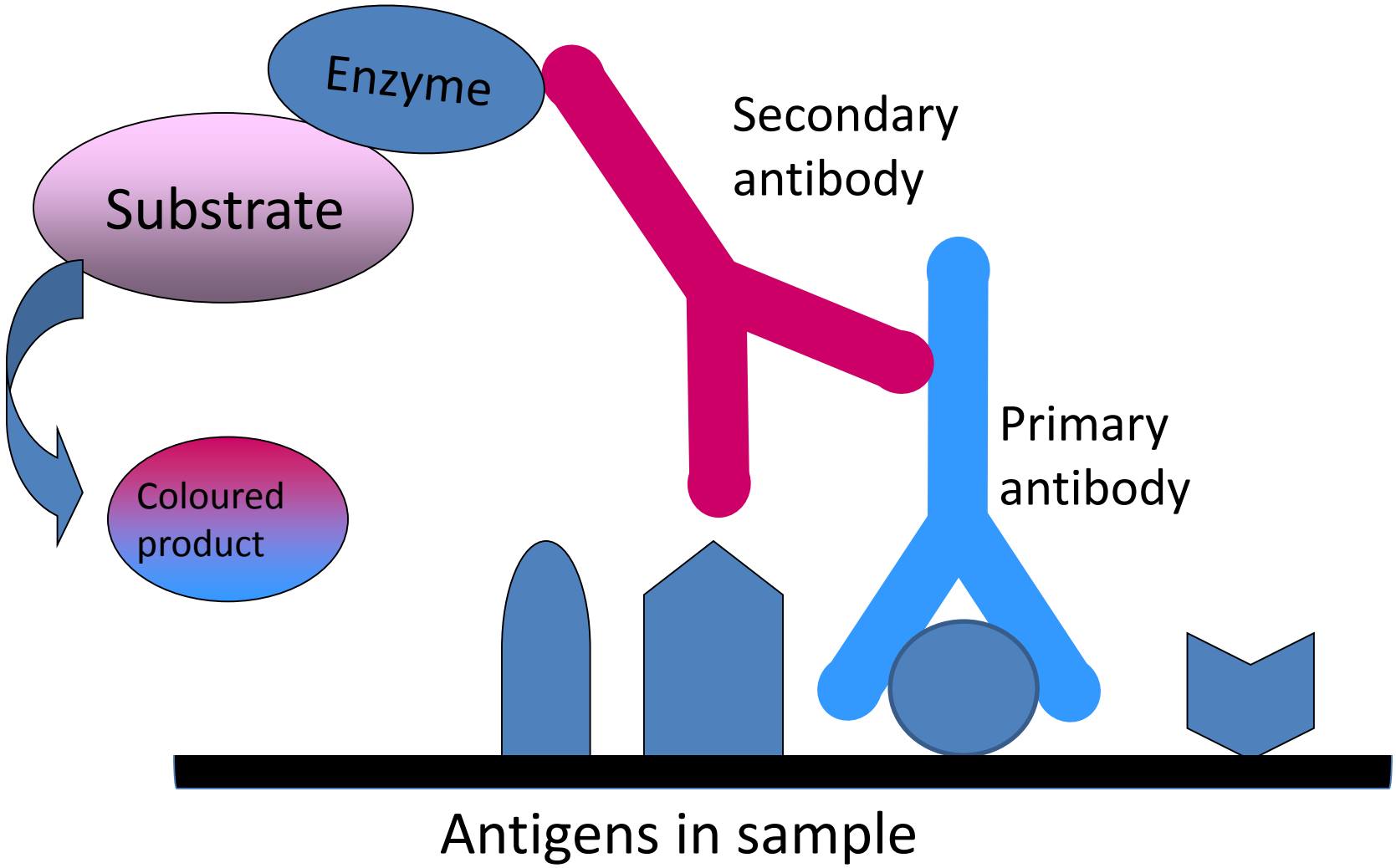


Direct ELISA

- Fast and eliminates possible non-specific binding of secondary antibody

- However, reactivity of primary antibody may be compromised conjugation (little signal amplification)

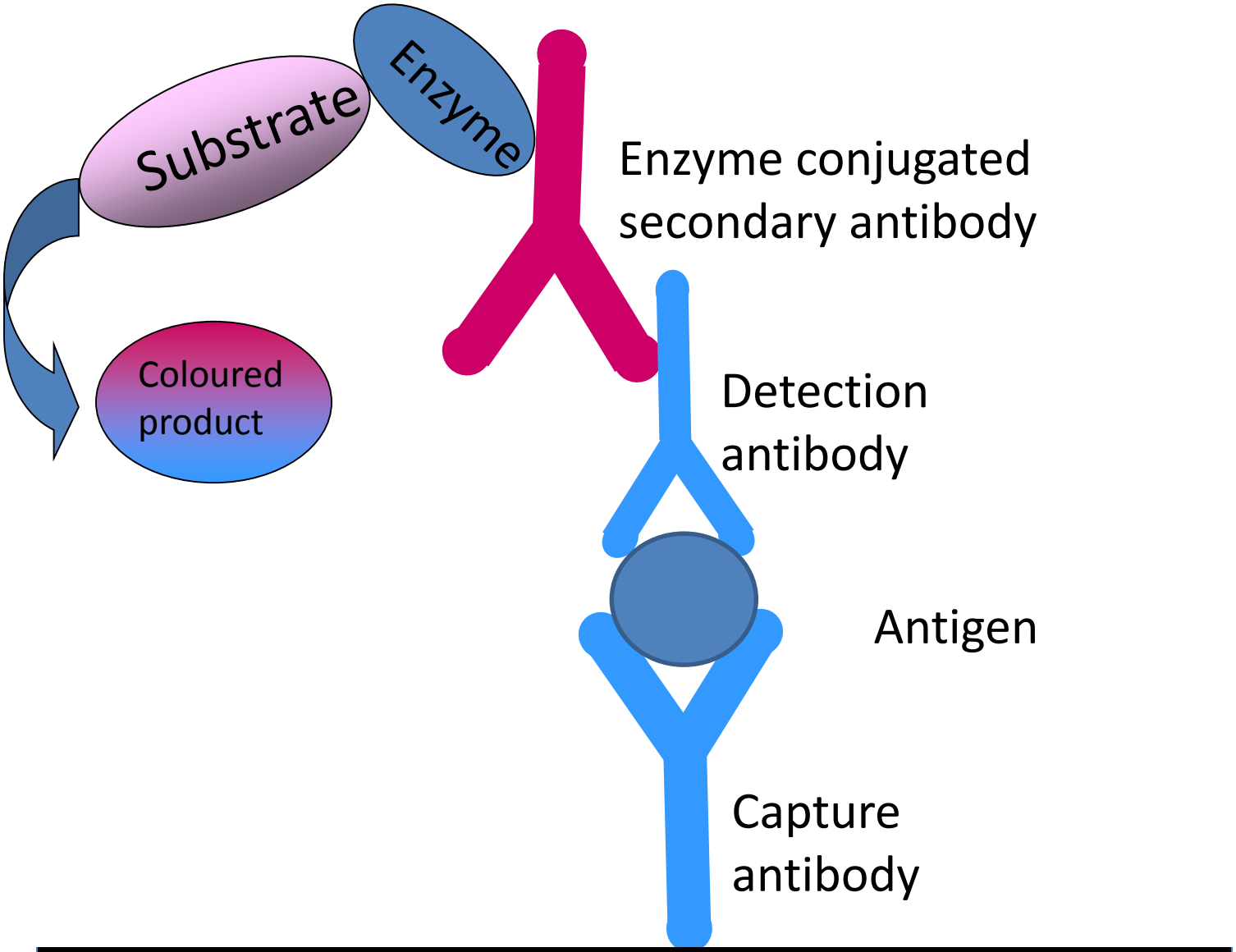
Indirect ELISA



Indirect ELISA

- ✓ Greater signal amplification
- ✓ But more time consuming and could get high background due to non-specific binding of the secondary antibody

Sandwich ELISA



Sandwich ELISA

- Very sensitive
- Highly specific
- But more time consuming and expensive

Blocking non-specific binding sites

Why do you need to block non-specific binding sites?

- Want non-reactive proteins to occupy protein-binding sites not bound with antigen, which might lead to antibody binding and high background signal
- Frequently used blocking agents are typically 2-5% BSA, non-fat dry milk, gelatin and some commercially available blocking kits

Methods

- Coat the plate and incubate for overnight
- Wash the plate 3 times
- Add the blocking (1hr)
- Add samples incubate for 2hrs
- Prepare positive and negative control
- Wash plate 3-5 times with washing buffer
- Add detection antibody (1hr)
- Wash plate 3-5 times with washing buffer

- Add 100µl of conjugate (anti-human-IgG-HRP) to each well and incubate for 30 min
- Wash plate 3-5 times with washing buffer
- Add 100µl substrate (tetramethylbenzidine; TMB) to each well and incubate for 15 min in the dark
- Add 50µl stop solution (0.5M H_2SO_4) to each well
- Read the optical density on an ELISA plate reader at 450nm

Enzyme conjugates

Possible enzymes used in the ELISA system are

1. alkaline phosphatase,
2. beta-galactosidase and
3. horseradish peroxidase

Substrate

- p-nitrophenyl phosphate (p-NPP) or TMB

Alkaline phosphatase (AP) catalyses the hydrolysis of p-nitrophenyl phosphate

(a colourless substrate) to phosphate and

p-nitrophenol (yellow at alkaline pH)

- The amount of p-nitrophenol equals amount of antibody present.