YAKIN DOĞU ÜNİVERSİTESİ

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DENEYSEL SAĞLIK BİLİMLERİ ARAŞTIRMA MERKEZİ
DESAM

Enzyme Linked Immunosorbent Assay (ELISA)

Dr Ferdiye Taner

Faculty of Medicine

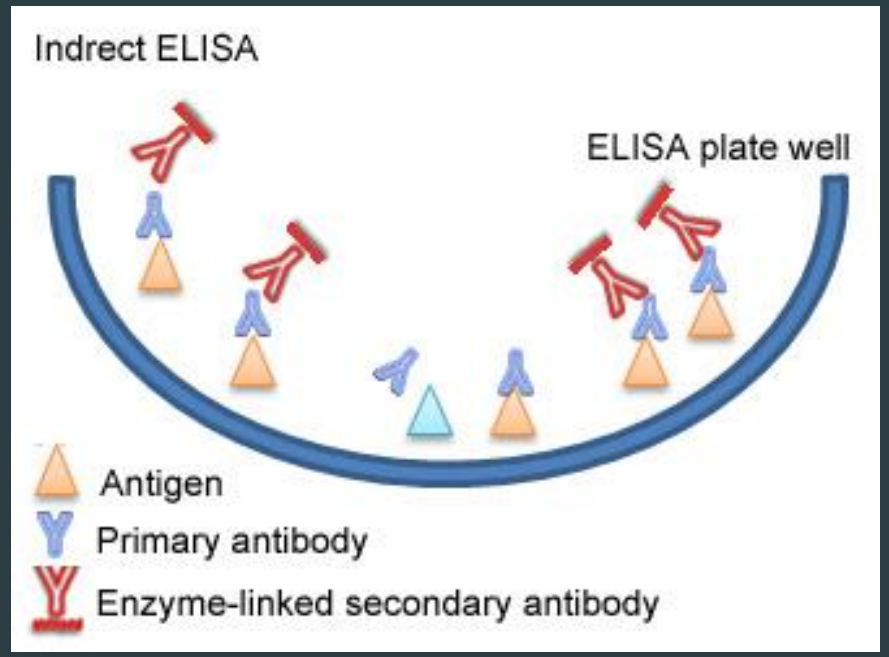
What is ELISA? (Enzyme-linked immunosorbent assay)

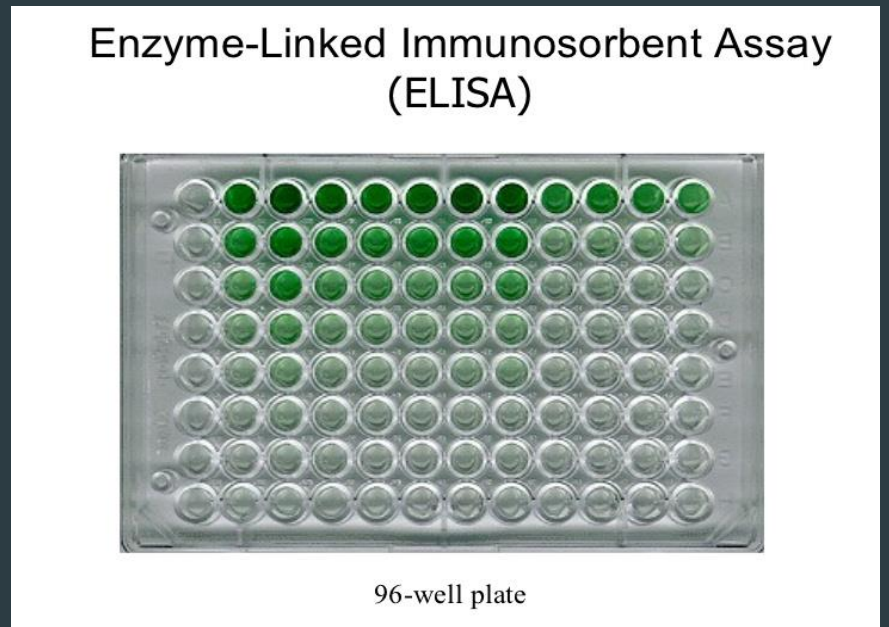
- ELISA is an antibody-based method
  - Indirect ELISA
  - Sandwich ELISA
  - ELISPOT
  - Competitive ELISA
  - Direct ELISA

- Designed to **quantitatively** or **quantitatively** detect a specific antigen (proteins, peptides, hormones) or an antibody in a sample

- Sample types:
  - Cell culture
  - Biological fluids (Plasma, Serum, Urine)
  - Purified recombinant protein in solution

Basic principle behind two-step ELISA



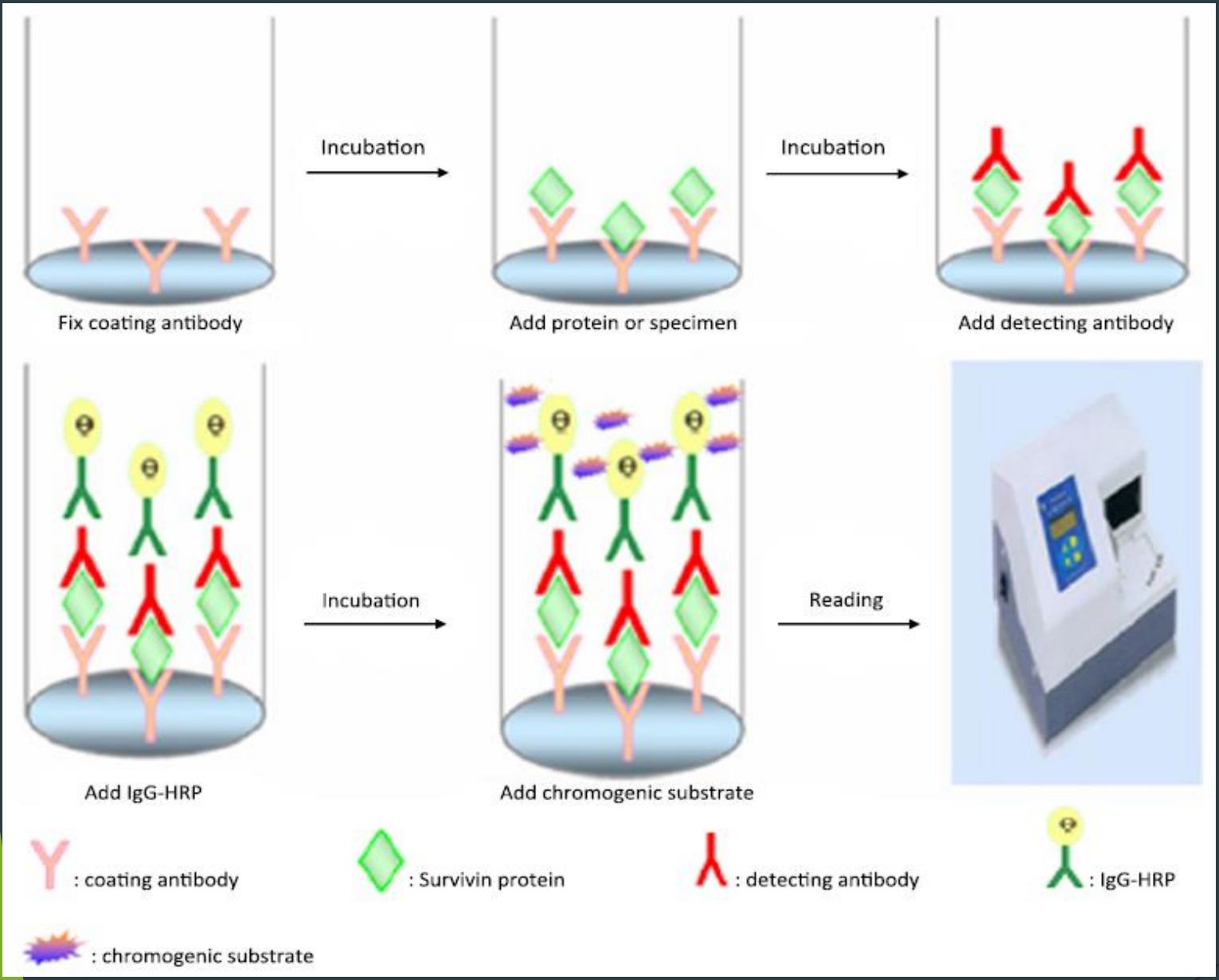


- Involves two binding process of primary antibody and labelled secondary antibody
  - **1.** 96-well plates are incubated with antigen
  - **2.** Antibody is added, sample is washed, any unbound antibody is washed off
  - **3.** Incubated with an enzyme-linked secondary antibody
  - **4.** A colourless chromogenic substrate is then added, a coloured derivative will form once the enzyme conjugate catalyses a reaction
  - **5.** The colour change is used to confirm the presence of an analyte both qualitatively and quantitatively

Basic principle behind two-step ELISA

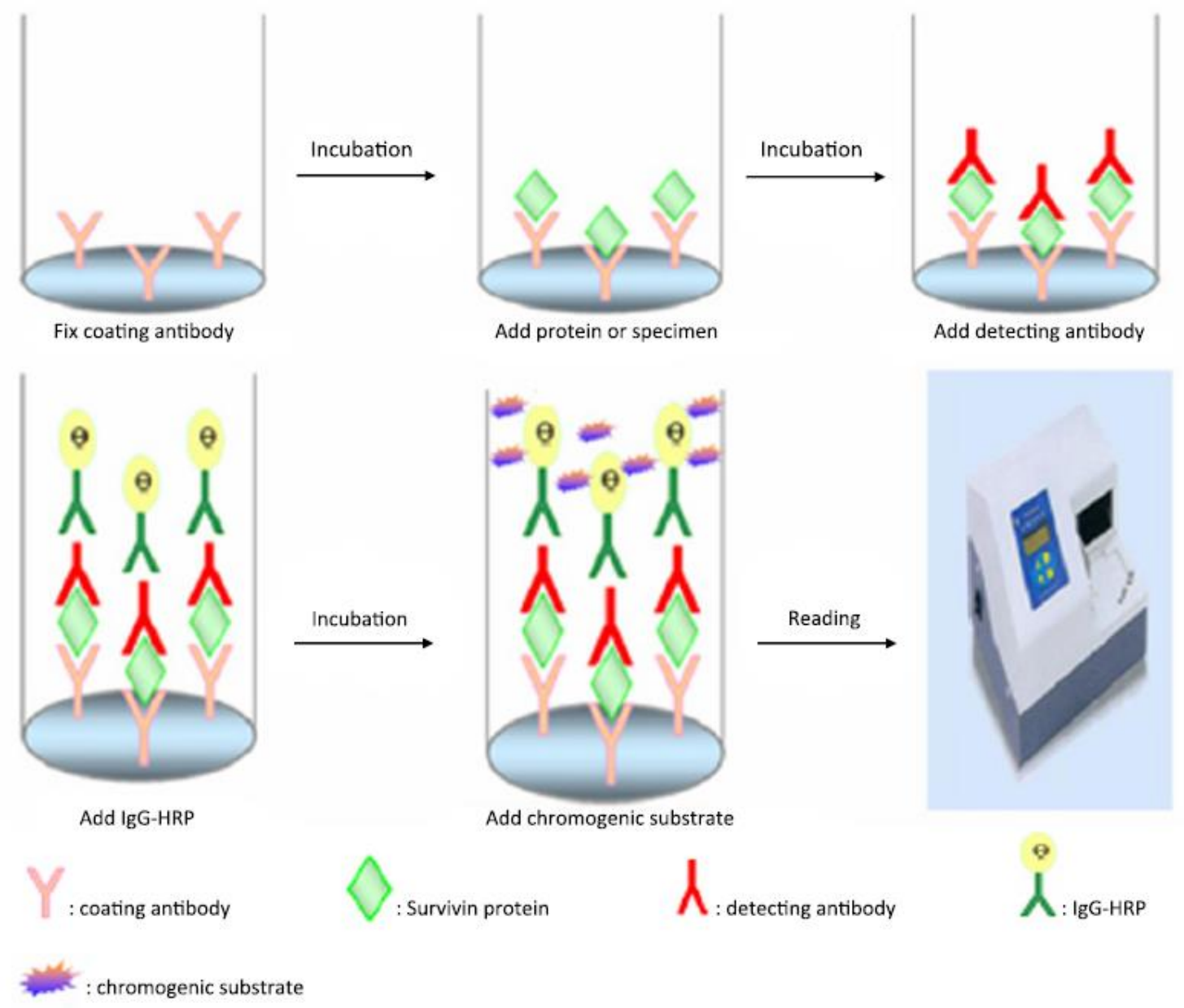
- https://youtu.be/RRbuz3VQ100

Basic principle behind a Sandwich ELISA



- **Quantifies antigens between two layers of antibodies**
- 1. Prepare surface to which capture antibody is bound
- 2. Apply the antigen –containing sample to the plate
- 3. Include a wash step, so that any unbound antigen(s) is removed
- 4. A specific antibody is then added which binds to the antigen
- 5. An enzyme-linked secondary antibody is added which binds specifically to the antibody's Fc region
- 6. Include a wash step to remove any unbound antibody-enzyme conjugates
- 7. Apply a chemical that is converted by the enzyme into colour of fluorescent

Basic principle behind a Sandwich ELISA



- In a technical sense, if the primary antibody is conjugated to an enzyme, a secondary antibody conjugated to an enzyme is not necessary
- This approach however is cost-efficient, as it prevents developing an enzyme-linked antibody for every antigen that may require detection
- This same enzyme-linked antibody may be used in different situations due to the fact that the antibody binds to the Fc region in other antibodies

Sandwich ELISA advantages

- The antigen/analyte is specifically captured by two antibodies and detected, therefore it is a highly specific approach

- It is an approach that is suitable for complex samples, since the antigen does not need to be purified from the sample prior to measurement

Basic principle behind a Sandwich ELISA

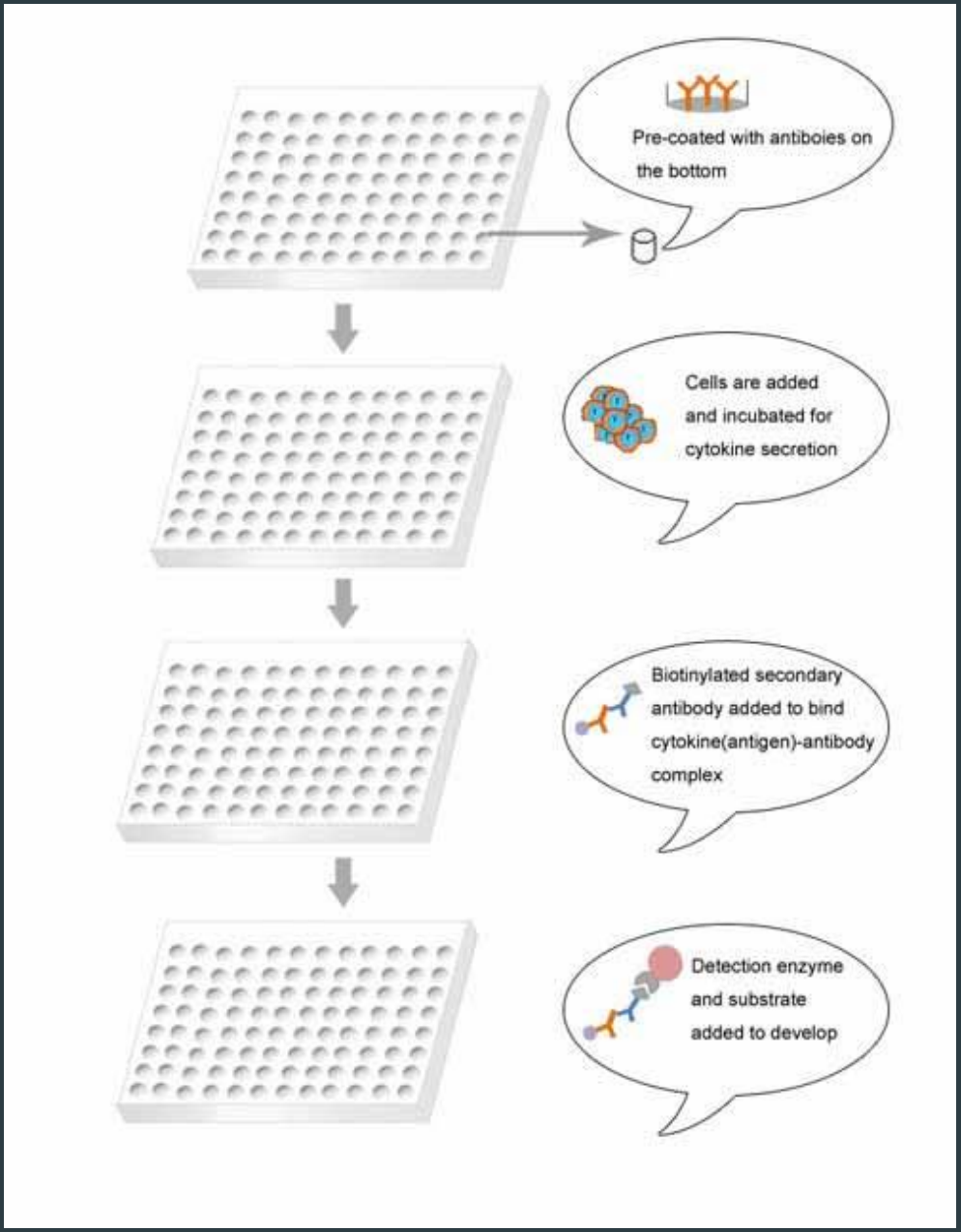
- https://youtu.be/6Ue1Hd3dyaQ

Is an ELISA assay suitable for your experiment?

- ELISA is a fast and an accurate assay that allows for either quantitative or qualitative detection of an analyte in a given sample

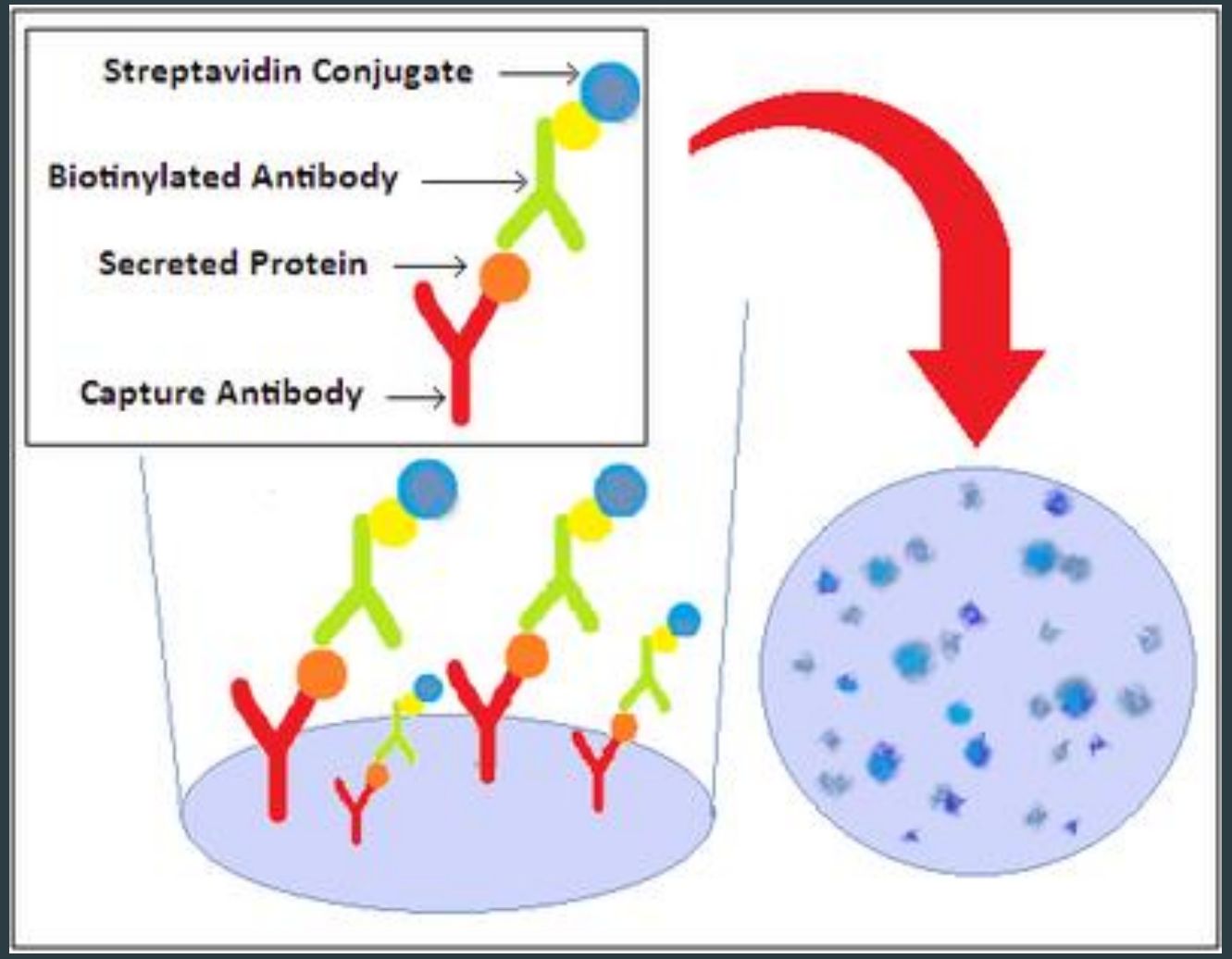
- The following criteria should be considered prior to performing an ELISA assay:
  - An antibody must be able to recognise the analyte of interest
  - The analyte needs to be present in an aqueous sample
  - The sample to be analysed should not contain any substance(s) that may interfere with antigen recognition or colour detection
  - There must be enough of the analyte present in the sample

Basic principle behind ELISpot

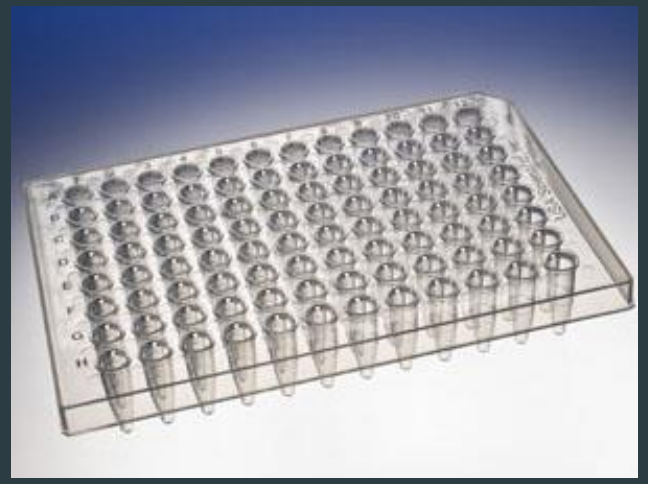


- Initially cells (T-cells) are cultured directly on the bottom of 96-well ELISpot plates or ones with PVDF or NC membranes which are pre-coated with specific primary antibodies to detect secreted cytokines
- T cells secret different cytokines. Rapidly cytokines are captured by antibodies on the membranes. After cells are washed off, captured cytokines are bond by biotin-conjugated secondary antibodies
- Enzyme-avidin combines with biotin and reveals colors with certain substrate. So far there will be visual spots on membranes. Every spot represents single cell that secrets certain cytokine. These cells are called spot forming cells, SFCs
- Positive ratio can be calculated by a divide of total cell number by spot number

Basic principle behind ELISpot



ELISA Devices



- Most plates are polystyrene
- 96-wells/8 rows
- Each well holds a volume of 350 µl

- 384 well and 1536 well plates are also available for high throughput screening

Multi-channel, 8- and 12-channels

ELISA Devices

Washer system

ELISA plate reader

ELISA advantages

High sensitivity

- The ELISA can be used to detect the presence of antigens that are recognised by an antibody or alternatively the approach can be used to detect for the presence of antibodies that recognise an antigen

Strong specificity

- Specificity of ELISA is because of the **selectivity** of the antibody or antigen