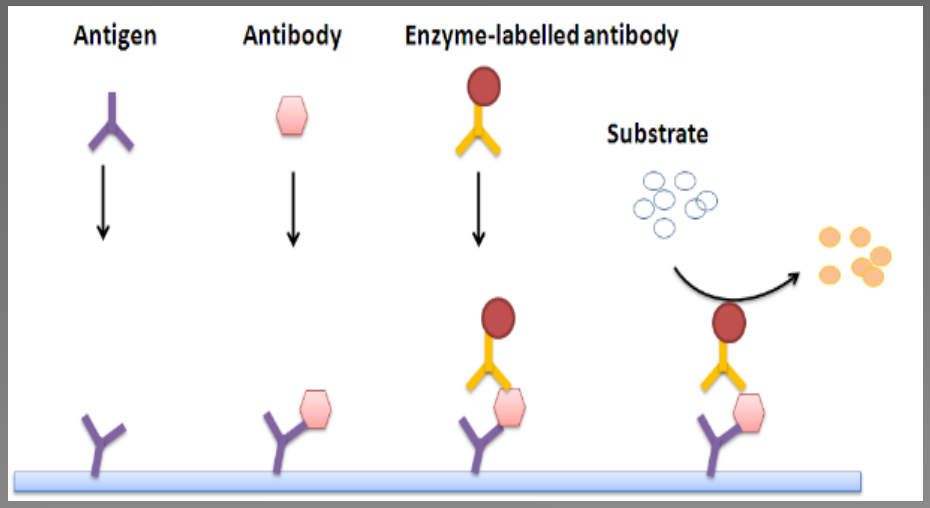
ELISA

Introduction



Introduction

- A sandwich ELISA measures antigen between two layers of antibodies (capture and detection antibody). The target antigen must contain at least two antigenic sites capable of binding to antibodies.
- Monoclonal or polyclonal antibodies can be used as the capture and detection antibodies in sandwich ELISA systems. Monoclonal antibodies recognize a single epitope that allows quantification of small differences in antigen. A polyclonal is often used as the capture antibody to pull down as much of the antigen as possible.
- Sandwich ELISAs remove the sample purification step before analysis and enhance sensitivity (2–5 times more sensitive than direct or indirect).

Introduction

- A capture antibody on a multi-well plate will immobilize the antigen of interest. This antigen will be recognized and bound by a detection antibody conjugated to biotin and streptavidin-HRP.

- ELISA stands for enzyme-linked immunosorbent assay, also often referred to as enzyme immunoassay (EIA). An ELISA, like other types of immunoassays, relies on antibodies to detect a target antigen using highly specific antibody-antigen interactions.

- In an ELISA assay, the antigen must be immobilized to a solid surface. This is done either directly or via the use of a capture antibody itself immobilized on the surface. The antigen is then complexed to a detection antibody conjugated with a molecule amenable for detection such as an enzyme or a fluorophore.

- An ELISA assay is typically performed in a multi-well plate (96- or 384-wells). The multi-well plate provides the solid surface to immobilize the antigen. Immobilization of the analytes facilitates separation of the antigen from the rest of the components in the sample. This characteristic makes ELISA one of the easiest assays to perform on multiple samples simultaneously.

Introduction

- The ELISA method is a benchmark for quantitation of antigens. ELISAs are adaptable to high-throughput screening because results are rapid, consistent and relatively easy to analyze. The best results have been obtained with the sandwich format, utilizing highly purified, pre-matched capture and detection antibodies. The resulting signal provides data which is very sensitive and highly specific. Ready-to-use ELISA kits are commercially available for hundreds of commonly investigated proteins and other biological molecules.

ELISA History

- 1798 - First demonstration of vaccination smallpox vaccination (Edward Jenner)
- 1890 - Demonstration of antibody activity against diphtheria and tetanus toxins. Beginning of humoral theory of immunity. (Emil von Behring) and (Shibasaburo Kitasato)
- 1900 - Antibody formation theory (Paul Ehrlich)
- 1938 - Antigen-Antibody binding hypothesis (John Marrack)
- 1948 - antibody production in plasma B cells
- 1959-1962 - Discovery of antibody structure
- 1960 - Radioimmunoassay was first described in a scientific paper by Rosalyn Sussman Yalow and Solomon Berson published in 1960
- 1966 - A technique to prepare something like immunosorbent to fix antibody or antigen to the surface of a container was published by Wide and Jerker Porath in 1966
- 1971 - Peter Perlmann and Eva Engvall at Stockholm University invented ELISA
- 1975 - Generation of the first monoclonal antibodies (George Kohler and Cesar Milstein)

Understand all the basic principles of ELISA and decide if this is the right technique for your experiments.

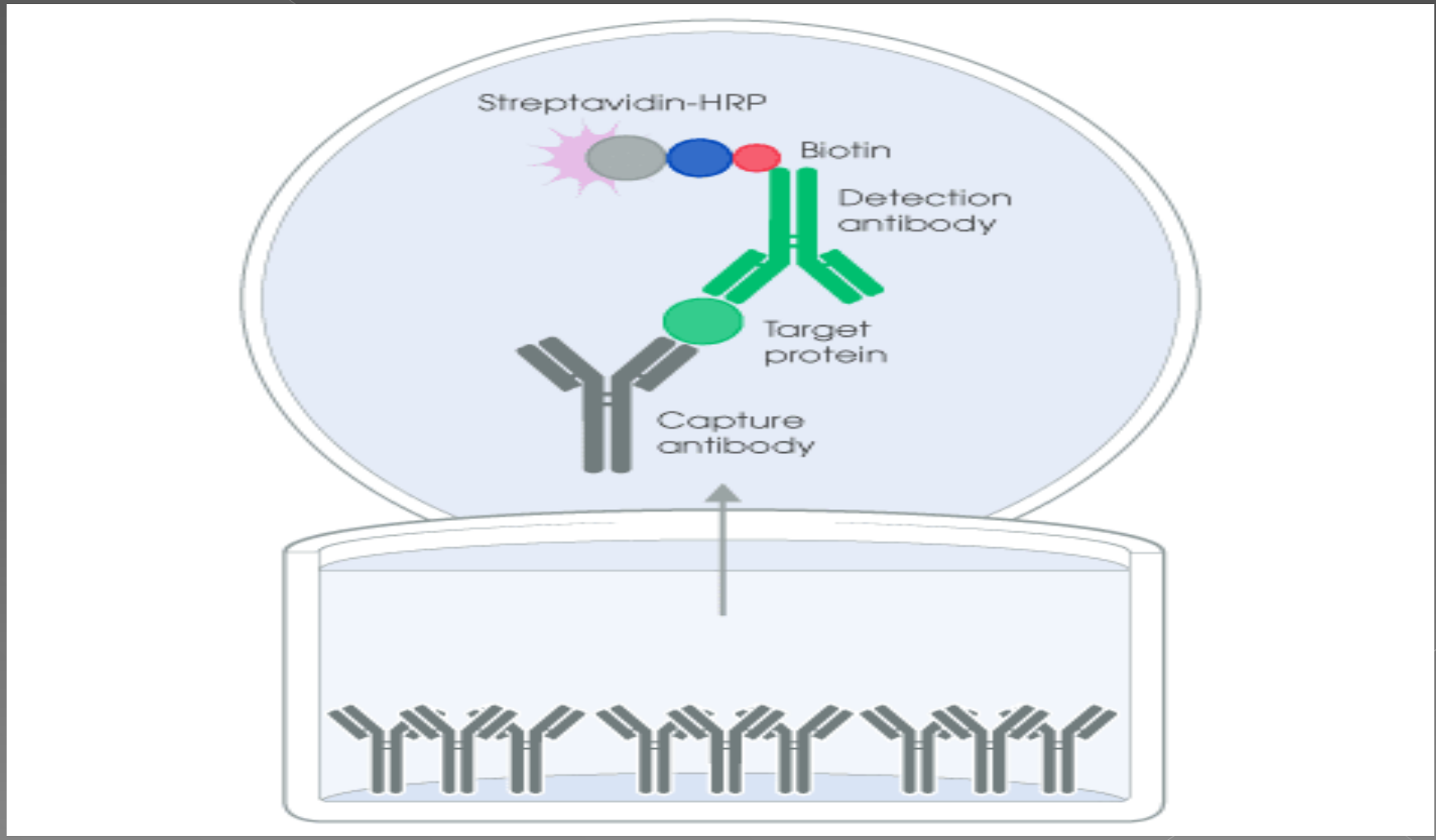


Figure 1. The basic setup of an ELISA assay.

ELISA Terms

- Solid phase

  Usually a microtiter plate well. Specially prepared ELISA plates are commercially available. These have an 8 × 12 well format and can be used with a wide variety of specialized equipment designed for rapid manipulation of samples including multichannel pipets.
- Adsorption

  The process of adding an antigen or antibody, diluted in buffer, so that it attaches passively to the solid phase on incubation. This is a simple way for immobilization of one of the reactants in the ELISA and one of the main reasons for its success.
- Washing

  The simple flooding and emptying of the wells with a buffered solution to separate bound (reacted) from unbound (unreacted) reagents in the ELISA. Again, this is a key element to the successful exploitation of the ELISA.

ELISA Terms

- Antigens
- A protein or carbohydrate that when injected into animals elicits the production of antibodies. Such antibodies can react specifically with the antigen used and therefore can be used to detect that antigen.
- Antibodies
- Produced in response to antigenic stimuli. These are mainly protein in nature. In turn, antibodies are antigenic.
- Antispecies antibodies
- Produced when proteins (including antibodies) from one species are injected into another species. Thus, guinea pig serum injected into a rabbit elicits the production of rabbit anti¨Cguinea pig antibodies.

ELISA Terms

- A substance that can react at low concentration as a catalyst to promote a specific reaction. Several specific enzymes are commonly used in ELISA with their specific substrates
- An enzyme that is attached irreversibly to a protein, usually an antibody. Thus, an example of antispecies enzyme conjugate is rabbit antiguinea linked to horseradish peroxidase.
- A chemical compound with which an enzyme reacts specifically. This reaction is used, in some way, to produce a signal that is read as a color reaction (directly as a color change of the substrate or indirectly by its effect on another chemical).

ELISA Terms

- A chemical that alters color as a result of an enzyme interaction with substrate.
- The process of stopping the action of an enzyme on a substrate. It has the effect of stopping any further change in color in the ELISA.
- Measurement of color produced in the ELISA. This is quantified using special spectrophotometers reading at specific wavelengths for the specific colors obtained with particular enzyme/chromophore systems. Tests can be assessed by eye.

References

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