NULIFE

AbChek ELISA Kits for Accurate Detection

ELISA (Enzyme-Linked Immunosorbent Assay) is a plate-based assay technique that allows for detection and quantification of an analyte present in a sample. ELISA uses the natural ability of antibodies to specifically bind to a target antigen, thus enabling detection and quantification of the antigen. Even if present in small concentrations, ELISA is able to detect multiple substances, including hormones, peptides, proteins and antibodies. Therefore, it is a valuable tool for diagnosis, industry and research

While several types of ELISA have been established, they all rely on the same principles:

Plate Coating

In most forms of ELISA, the plate can be coated with a primary capture antibody or antigen, These are immobilized directly to the surface of Polystyrene microplate well.

Plate Blocking

In this step, all the surface-binding sites present in the coated plates are saturated by adding a protein or molecule that does not interfere with the assay that is being carried out. Blocking the surface area is essential to obtain reliable results as the binding capacity of the plate is usually higher than the amount of antigen or antibody coated.

Sample and Standard Incubation

Samples and standards are added to the plate at the required dilutions, which will depend on the sample type and the analyte to itself. The standard has a known concentration, and it is used as a reference to allow for the calculation of the sample concentrations.

Antibody Incubation

The primary antibody is added to the wells and incubated. The primary antibody binds specifically to the target antigen. Incubation time and temperature are crucial for optimal binding (commonly 1-2 hours at room temperature or overnight at 4°C).

Detection and signal measurements

Signal detection and measurement varies according to the tag used. Colorimetric ELISA and CLIA typically use HRP or AP, and their corresponding substrate. The signal is then detected using a standard absorbance plate reader, which enables direct visualization and high reproducibility between plates. If the ELISA or CLIA has been accurately developed, the signal produced by the substrate will be proportional to the concentration of the antigen captured. For fluorescent tags, however, a fluorimeter that produces the appropriate excitation wavelength is required. This method also allows high reproducibility. Yet, it normally requires the use of black micro titer plate.

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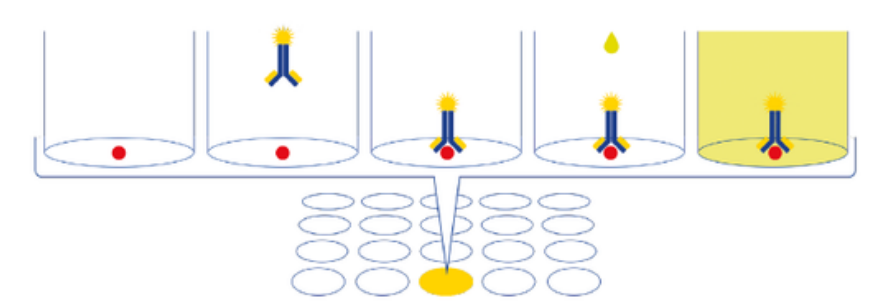
Direct ELISAs

Direct ELISAs are considered the simplest form of ELISA.

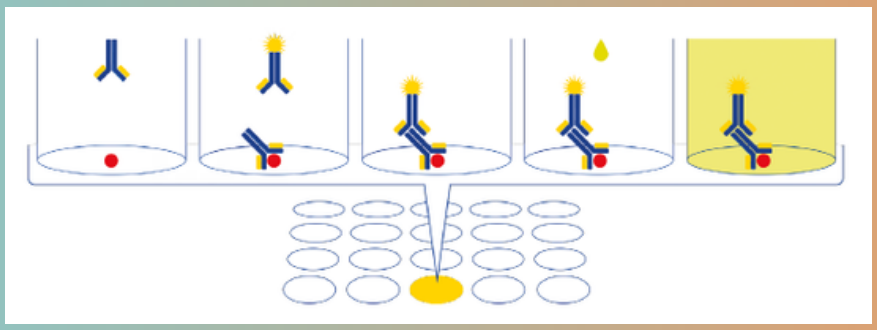
The sample is coated onto the plate either directly. Detection is via a labelled primary antibody which produces a colour change upon the addition of the substrate. Direct ELISAs are beneficial as no secondary antibody is required thus preventing cross reactivity between antibodies. This ELISA can also use a labelled antigen to detect an antibody coated onto the plate. As Direct ELISAs do not include a secondary antibody signal amplification step, they are less sensitive compared to Indirect ELISAs.



Indirect ELISAs

Indirect ELISAs use a secondary antibody conjugated to an enzyme to detect the primary antibody. The analyte is first coated onto the plate before primary antibody is added which will specifically bind to the analyte. Labelled secondary antibody is then introduced which will bind the primary antibody. The addition of substrate results in a colour change, the intensity of which correlates to the concentration of analyte.

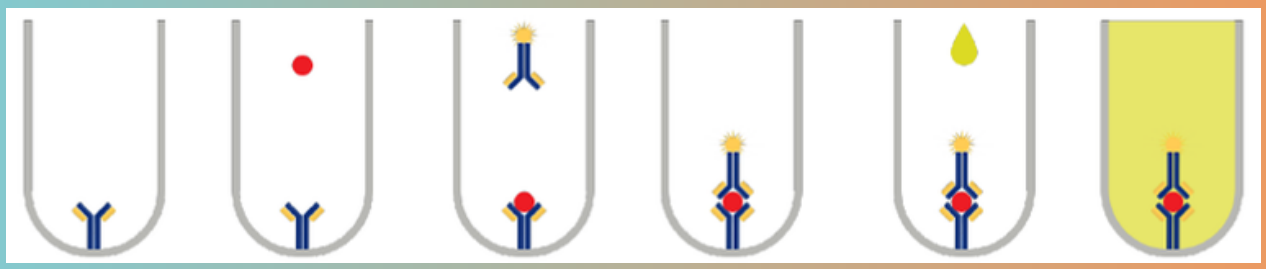
This type of ELISA has increased sensitivity due to the amplification effect of using a secondary antibody, as several secondary antibodies may bind onto the primary antibody. This type is usually less expensive because the primary antibody doesn't need to be conjugated, and secondary antibodies are inexpensive.



Sandwich ELISAs

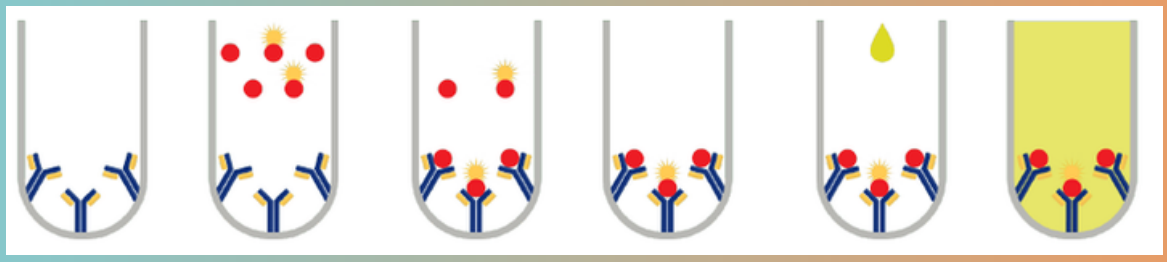
Sandwich ELISA is the most common type of ELISA. As the name suggests, the antigen of interest is sandwiched between two antibodies either directly or indirectly. This type of assay requires the use of matched pairs of antibodies that have been tested together to ensure that they bind different and non-overlapping epitopes of an antigen, thus preventing the antibodies binding to the same site. The capture antibody is coated on the surface of the plate and binds to the target antigen after the sample is added. The detection antibody, on the other hand, binds to the target antigen if it is has bound to the capture antibody and enables subsequent measurements either directly or indirectly.

Sandwich ELISA shows greater sensitivity and specificity as two antibodies are used to detect a single antigen. In general, it is a useful tool to analyse complex samples as they do not need to be purified prior to the assay. Similarly, sandwich ELISA is particularly useful when samples are suspected to have low concentrations of the antigen of interest.



Competitive ELISAs

Competitive ELISAs mainly used to identify small molecules such as lipids, hormones, and small peptides. This assay follows the principle that the antigen of interest and a conjugated version of the same antigen will compete for the binding sites present on the antibody. Therefore, the higher the sample antigen concentration, the weaker the signal output will be. The signal output is then inversely proportional to the amount of antigen in the sample. The labelled antigen will compete for binding to the antibody pre-coated to the wells. If more of the antigen in the sample is able to bind to, more of the labelled antigen will be removed following the wash cycles, and consequently, lower absorbance values will be obtained.



Reliable, Reproducible Results

ELISA results can be either qualitative or quantitative. Qualitative results provide either a positive or negative result for the sample by comparing the sample to known positive or negative controls. In quantitative ELISA, the absorbance signal observed the absorbance signal will be proportional to the concentration of the colourimetric tag and consequently, to the antigen. The concentration of the target in samples can be interpreted using a standard curve with known concentrations of the target.

Standard curves are produced by serial dilution of a known concentration of antigen or antibody. The optical density (OD) of the standard concentrations are measured in parallel with samples in the assay. Sample concentrations can then be quantified by interpolation of the respective sample OD on the standard curve. As sample concentration is determined using interpolation, sample concentration must be within the bounds of the standard curve for accurate calculation. Linear interpolation can often be used when the standard curve is plotted using log-linear scale. For standards plotted using a linear scale, a 2nd order polynomial or 4-parameter logistic regression is most commonly used.

Nulife Products

1. AbChek Human Asprosin ELISA Kit
2. AbChek Rat Tumor Necrosis Factor (TNF) ELISA Kit
3. AbChek Human Heme Oxygenase 1 (HMOX1) ELISA Kit
4. AbChek Human MERS-CoV IgG ELISA Kit
5. AbChek Human NEFL ELISA Kit
6. AbChek Human Fibulin 2 (FBLN2) ELISA Kit
7. AbChek Human Selenoprotein P / SEPP1 (SELENOP) ELISA Kit
8. AbChek Human S100 Calcium-binding Protein A10 (S1OOA10) ELISA Kit
9. AbChek Epinephrine/Adrenaline ELISA Kit
10. AbChek Human Torque Teno Virus (TTV) ELISA Kit

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