ELISA

ELISA (enzyme-linked immunosorbent assay) is a plate-based assay technique designed for detecting and quantifying peptides, proteins, antibodies and hormones. In an ELISA, an antigen must be immobilized to a solid surface and then complexed with an antibody that is linked to an enzyme. Detection is accomplished by assessing the conjugated enzyme activity via incubation with a substrate to produce a measureable product. The most crucial element of the detection strategy is a highly specific antibody-antigen interaction.

ELISAs are typically performed in 96-well (or 384-well) polystyrene plates, which will passively bind antibodies and proteins. It is this binding and immobilization of reagents that makes ELISAs so easy to design and perform. Having the reactants of the ELISA immobilized to the microplate surface makes it easy to separate bound from non-bound material during the assay. This ability to wash away nonspecifically bound materials makes the ELISA a powerful tool for measuring specific analytes within a crude preparation.

ELISA Basics/ELISA Principle

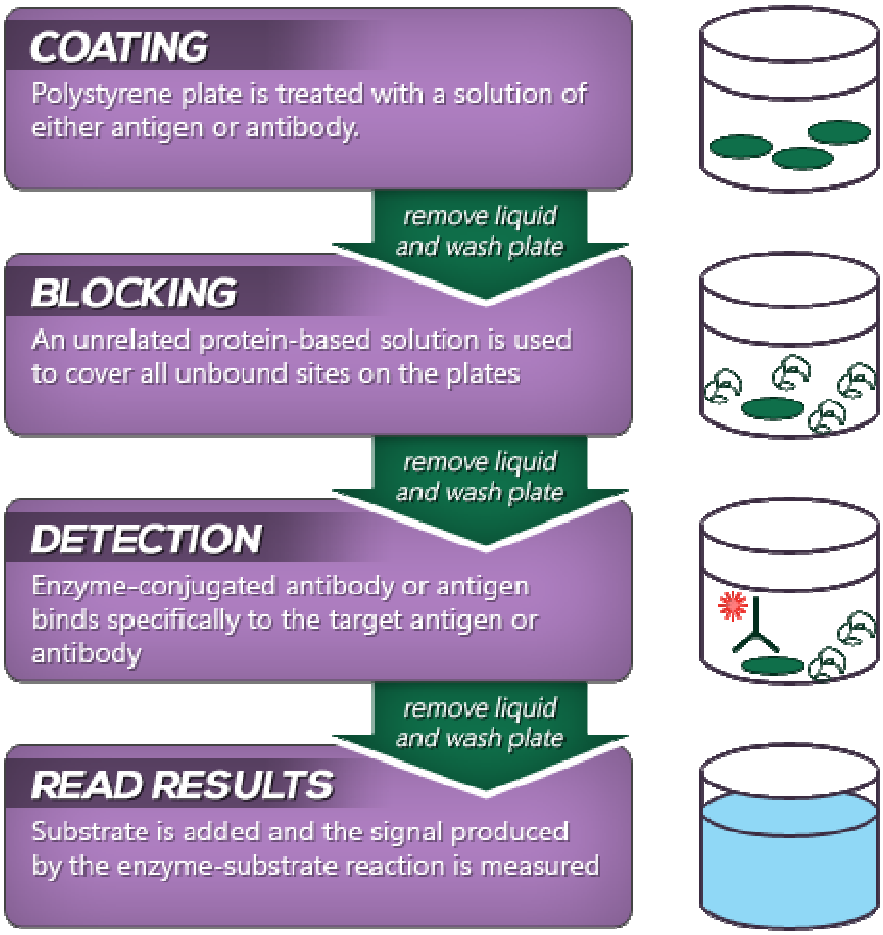
Enzyme-linked immunosorbent assays (ELISA) principles are very similar to other immunoassay technologies. ELISA's rely on specific antibodies to bind the target antigen, and a detection system to indicate the presence and quantity of antigen binding. In order to maximize the sensitivity and precision of the assay, the plate must be carefully coated with high-affinity antibodies – a process that Boster Bio has mastered.

General ELISA Procedure

Unless you are using a kit with a plate that is pre-coated with antibody, an ELISA begins with a **coating** step, in which the first layer, consisting of a target antigen or antibody, is adsorbed onto a 96-well polystyrene plate. This is followed by a **blocking** step in which all unbound sites are coated with a blocking agent. Following a series of washes, the plate is **incubated with enzyme-conjugated antibody**. Another series of washes removes all unbound antibody. A **substrate** is then added, producing a calorimetric signal. Finally, the plate is **read**.

Because the assay uses surface binding for separation, several washes are repeated in each ELISA step to remove unbound material. During this process, it is essential that excess liquid is removed in order to prevent the dilution of the solutions added in the next assay step. To ensure uniformity, specialized plate washers are often used.

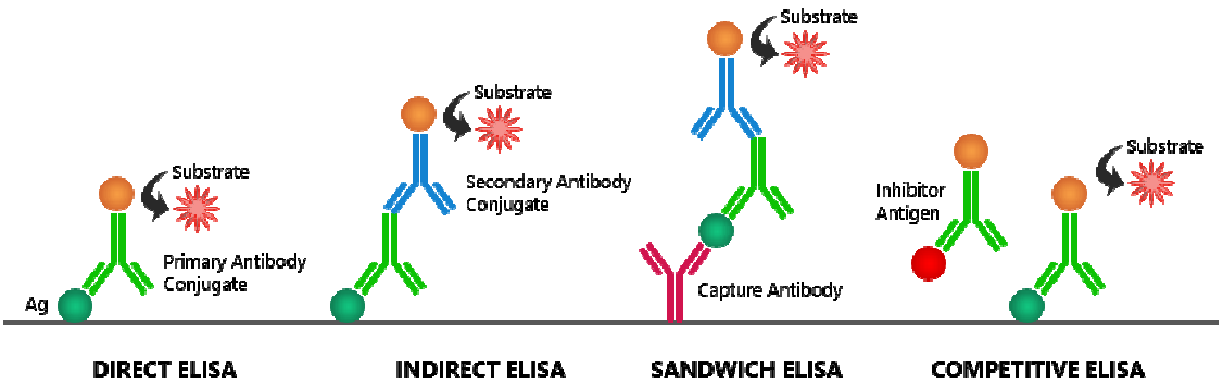
ELISAs can be quite complex and include multiple intervening steps, especially when measuring protein concentration in heterogeneous samples such as blood. The most complex and varying step in the overall process is detection, where multiple layers of antibodies can be used to amplify signal.



Four Most Common ELISA Types

ELISAs can be performed with a number of modifications to the basic procedure: direct, indirect, sandwich or competitive. The key step, immobilization of the antigen of interest, can be accomplished by direct adsorption to the assay plate or indirectly via a capture antibody that has been attached to the plate. The antigen is then detected either directly (enzyme-labeled primary antibody) or indirectly (enzyme-labeled secondary antibody). The detection antibodies are usually labeled with alkaline phosphatase (AP) or horseradish peroxidase (HRP). A large selection of substrates is available for performing the ELISA with an HRP or AP conjugate. The choice of substrate depends upon the required assay sensitivity and the instrumentation available for signal-detection (spectrophotometer, fluorometer or luminometer).

Among the standard assay formats discussed and illustrated below, where differences in both cpture and detection were the concern, it is important to differentiate between the particular strategies that exist specifically for the detection step. However an antigen is captured to the plate (by direct adsorption to the surface or through a pre-coated "capture" antibody, as in a sandwich ELISA), it is the detection step (as either direct or indirect detection) that largely determines the sensitivity of an ELISA.



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- ## Direct ELISA principle

  For direct detection, an antigen coated to a multi-well plate is detected by an antibody that has been directly conjugated to an enzyme. This detection method is a good option if there is no commercially available ELISA kits for your target protein.

  - **Advantages**
    - Quick because only one antibody and fewer steps are used.
    - Cross-reactivity of secondary antibody is eliminated.
  - **Disadvantages**
    - Cell Smear: Adhere non-adherent cells on coverslip with chemical bond
    - Immunoreactivity of the primary antibody might be adversely affected by labeling with enzymes or tags.
    - Labeling primary antibodies for each specific ELISA system is time-consuming and expensive.
    - No flexibility in choice of primary antibody label from one experiment to another.
    - Minimal signal amplification.
- ## Indirect ELISA principle

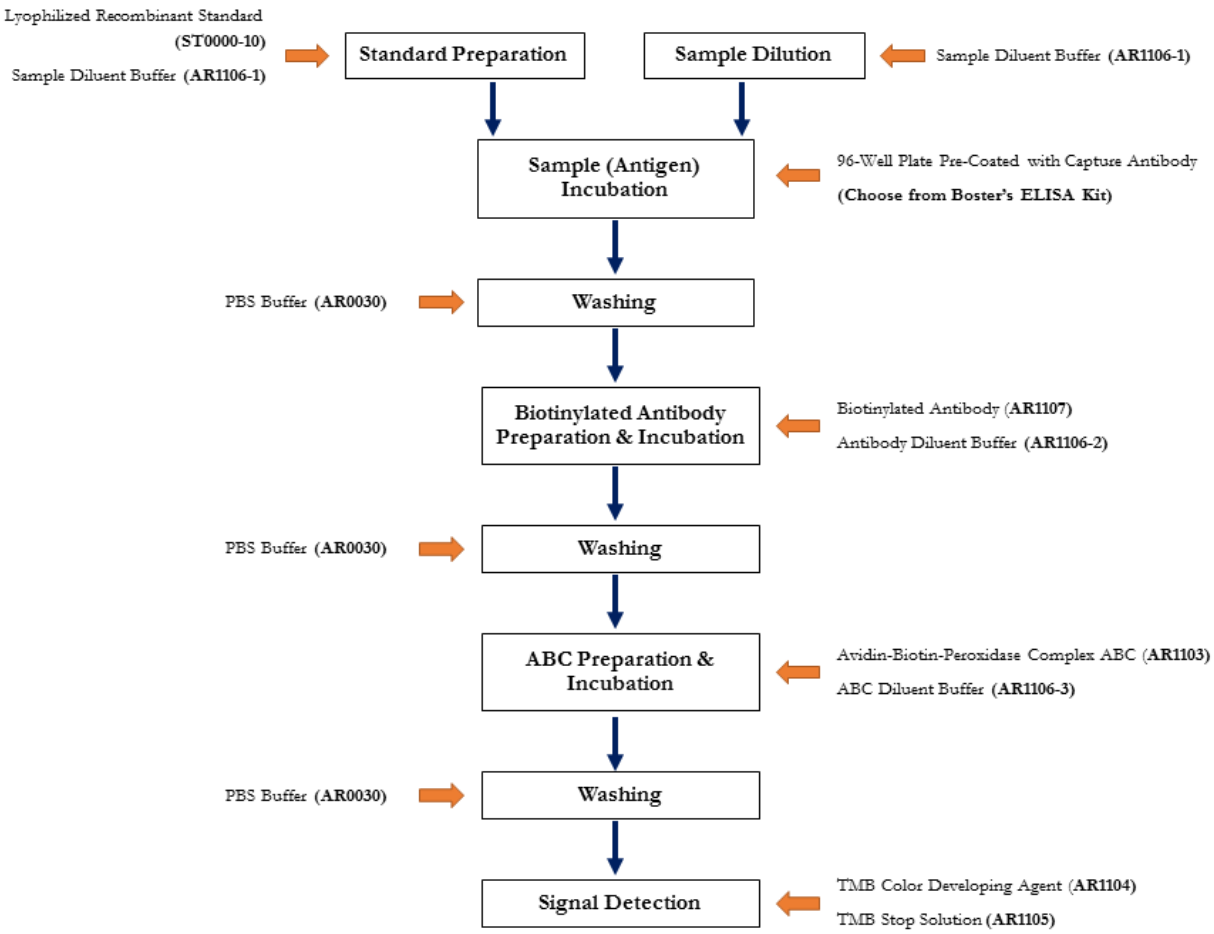
  For indirect detection, the antigen coated to a multi-well plate is detected in two stages or layers. First an unlabeled primary antibody, which is specific for the antigen, is applied. Next, an enzyme-labeled secondary antibody is bound to the first antibody. The secondary antibody is usually an anti-species antibody and is often polyclonal. The indirect assay, the most popular format for ELISA, has the advantages and disadvantages:

  - **Advantages**
    - A wide variety of labeled secondary antibodies are available commercially.
    - Versatile because many primary antibodies can be made in one species and the same labeled secondary antibody can be used for detection.
    - Maximum immunoreactivity of the primary antibody is retained because it is not labeled.

        - Sensitivity is increased because each primary antibody contains several epitopes that can be bound by the labeled secondary antibody, allowing for signal amplification.
    - **Disadvantages**
        - Cell Smear: Adhere non-adherent cells on coverslip with chemical bond
        - Cross-reactivity might occur with the secondary antibody, resulting in nonspecific signal.
        - An extra incubation step is required in the procedure.
- **Sandwich ELISA principle**

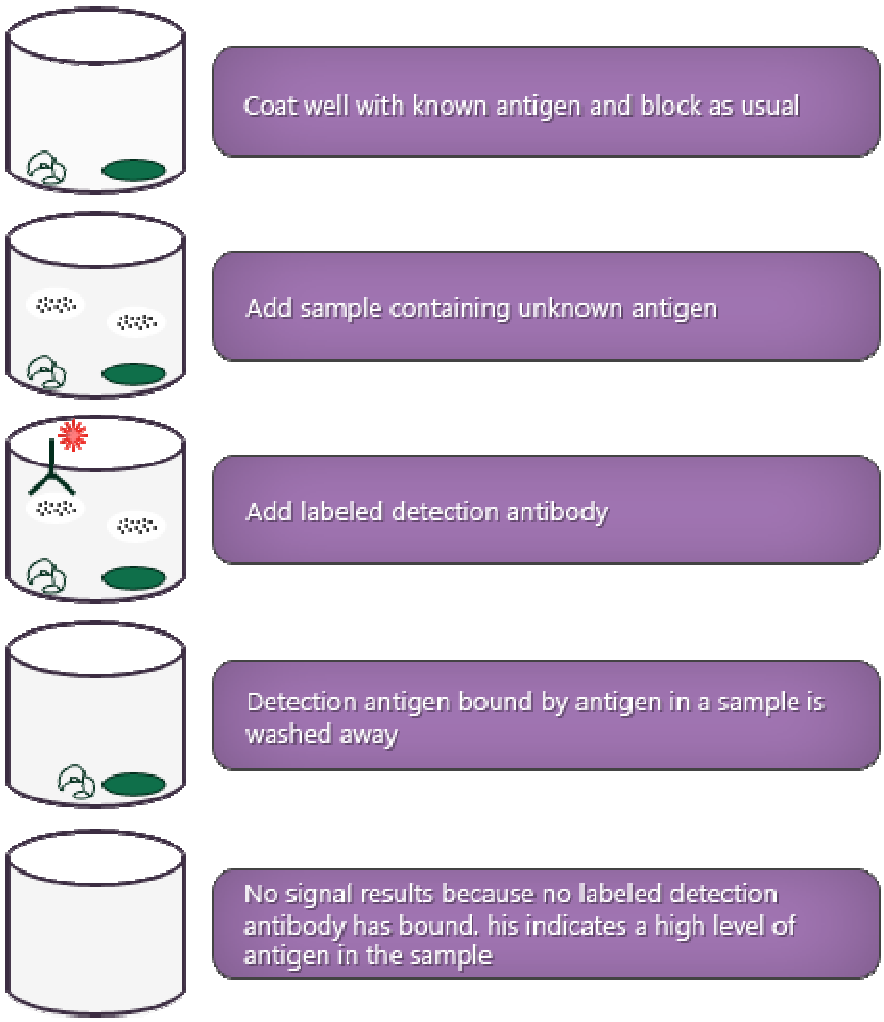
Sandwich ELISAs typically require the use of matched antibody pairs, where each antibody is specific for a different, non-overlapping part (epitope) of the antigen molecule. A first antibody (known as capture antibody) is coated to the wells. The sample solution is then added to the well. A second antibody (known as detection antibody) follows this step in order to measure the concentration of the sample. This type of ELISA has the following advantages:

- High specificity: the antigen/analyte is specifically captured and detected
- Suitable for complex (or crude/impure) samples: the antigen does not require purification prior to measurement
- Flexibility and sensitivity: both direct or indirect detection methods can be used



Competitive ELISA principle

This ELISA kit is of competitive format. Competitive ELISA, also known as inhibition ELISA, is a surface/plate based assay, where the plate is coated with capture antibodies reactive to the molecule of interest. The sample (containing native molecule of interest) and enzyme conjugated recombinant protein (the competing molecule) are added to the coated wells. Since the amount of enzyme conjugated molecule in each well is constant, the level of native molecule in the sample will determine the binding ratio of enzyme conjugated molecule vs. native molecule. After an incubation period, any unbound antibody is washed off. Enzyme substrate (for example, TMB for HRP) is added to each well and will be transformed into a blue precipitate, the amount of which is linearly proportional to the amount of enzyme in the well. The precipitate is then turned into yellow by adding the acid stop solution and the concentration of yellow precipitate is read at 450nm for light absorbance (O.D. value). The O.D. is then used to calculate the amount of molecule of interest in each well, by comparing each sample well against the standard curve. The standard curve is generated using the same principle but instead of adding samples, a series of recombinant molecules with known concentrations are added to 6-8 wells.



Summary of Key Steps in Different ELISA Types

	Indirect	Direct	Sandwich	Competitive
Capture Ab Coating	✗	✗	✓	✗
Antigen Coating	✓	✓	✗	✓

	Indirect	Direct	Sandwich	Competitive
Blocking	✓	✓	✓	✓
Sample (Antigen) Incubation	✗	✗	✓	✓
Primary Ab Incubation	✓	✓	✓	✓
Secondary Ab Incubation	✓	✗	✓	✓
Substrate Prep	✓	✓	✓	✓
Signal Detection	✓	✓	✓	✓
Data Analysis	✓	✓	✓	✓

ELISA Data Interpretation

The ELISA assay yields three different types of data output:

1. **Quantitative:** ELISA data can be interpreted in comparison to a standard curve (a serial dilution of a known, purified antigen) in order to precisely calculate the concentrations of antigen in various samples.
2. **Qualitative:** ELISAs can also be used to achieve a yes or no answer indicating whether a particular antigen is present in a sample, as compared to a blank well containing no antigen or an unrelated control antigen.
3. **Semi-Quantitative:** ELISAs can be used to compare the relative levels of antigen in assay samples, since the intensity of signal will vary directly with antigen concentration.

ELISA data is typically graphed with optical density (or fluorescence) vs concentration to produce a sigmoidal curve as shown below. Known concentrations of antigen are used to produce a standard curve and then this data is used to measure the concentration of unknown samples by comparison to the linear portion of the standard curve. In fact, it is the relatively long linear region of the curve that makes the ELISA results accurate and reproducible. The unknown concentration can be determined directly on the graph or with curve fitting software which is typically found on ELISA plate readers.