Eight tips for ensuring a successful ELISA

Enzyme-linked immunosorbent assays (ELISAs) are a type of multiwell plate-based technique used to determine the concentrations of antibodies, antigens or other proteins in a biological sample using chromogenic, fluorescent or luminescent readouts.[1] Since their development in the 1960s, they have become a staple in many diagnostic and biomedical research labs, demonstrating specificity, sensitivity and reproducibility across a range of applications. Common examples include the detection of various infectious diseases and pregnancy tests.[2,3]

While there are many advantages to using ELISAs, they must be appropriately set-up and optimized to ensure reliable results. This guide will provide an overview of the different types of ELISA and highlight tips for ensuring the success of your ELISA experiments.

1. Choosing the right assay

ELISAs can be used as diagnostic, monitoring and quality control tools across medicine and the food industry.[4] As outlined in Table 1, there are several types of ELISA to choose from depending on the research goal. The target of detection in an ELISA may be an antigen (e.g. if aiming to detect the presence of a virus) or may be an antibody (e.g. if aiming to detect an immune response to an antigen). For simplicity, the examples below illustrate antigen detection, however the same principal is true for antibody detection. This section will provide an overview of each type.

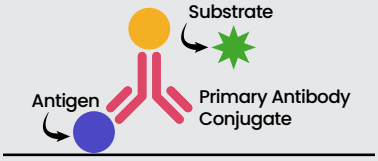
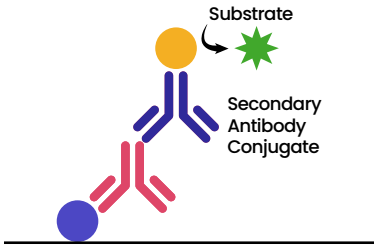
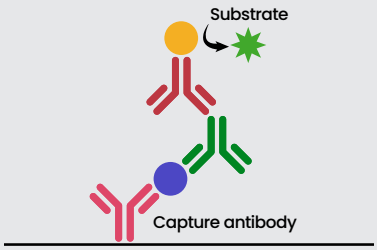
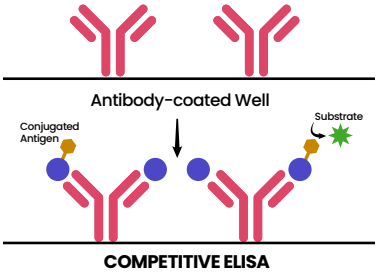
Type	Key points	Advantages	Disadvantages
 DIRECT ELISA	Binds antigens, including the desired target, in a sample directly to the plate. An enzyme-conjugated antibody is then added as a probe for the desired analyte.	Only one antibody is used, so cross-reactivity is not a concern Rapid	Low sensitivity Non-specific binding of antigens so background may be high
 INDIRECT ELISA	Binds antigens, including the desired target, in the sample to the plate. However, it involves two antibodies; a primary antibody and a secondary conjugated antibody.	High sensitivity	There is a risk of antibody cross-reactivity Non-specific binding of sample antigens so background may be high
 SANDWICH ELISA	The target is bound between a capture antibody (for antigen detection) or capture protein (for antibody detection) and the conjugated detecting antibody, creating a “sandwich”.	Highly sensitive and specific	Choosing the right antibody pair can be time-consuming
 COMPETITIVE ELISA	Involves competition between the binding of the sample antigen and conjugated antigen to a specific amount of antibody. The more antigen in the sample, the less conjugated antigen binds and the lower the assay signal.	Rapid Requires little/ no sample pre-processing Useful for small targets that cannot easily be bound with two antibodies	Low specificity

Table 1: The advantages and disadvantages of the different types of ELISAs.

2. Planning ahead

Advanced planning can help the workflow run efficiently and reduce the likelihood of errors and retests; therefore, it is necessary to plan all components of the workflow thoroughly before it begins. Plate and assay design are important planning considerations. The factors that need to be determined include: the number of samples to be tested, the number of replicates and the number of plates/kits required. Including sample replicates for ELISAs is important as this will highlight anomalous results. Triplicates are preferred to increase reliability, but duplicates can be used if necessary. Controls also need to be factored into the protocol, as this will indicate if the assay has been successful, remained within the dynamic range and enable inter-assay comparison and correction. Using a plate map to plan your ELISA assay will also help to reduce any sample location mistakes.

When running multiple plates or multiple assays using different reagents, all plates need to be clearly labeled and reagents segregated to avoid mix-ups. Additionally, all the reagents must be thoroughly thawed (if required), mixed and in-date before use and checked that any light sensitive reagents have not deteriorated. Another factor to consider, especially for a sandwich ELISA is the species from which the primary and secondary antibodies have been derived. When working with antibody pairs, it is essential that they are tested to find the most optimal signal and minimize any cross-reactivity. Many commercially available kits provide the antibodies, however, if a project involves a novel target, then it is important to test a range of antibodies against known and unknown samples. When optimizing an assay, try different antibody dilutions to increase the sensitivity of detection while minimizing cross reactivity and background noise. Trying a range of sample dilutions can also assist in this process.

Blocking is another crucial part of the workflow that reduces background signal and minimizes false-positive results. A good blocking buffer will reduce non-specific binding, while not (or only minimally) reacting with the antigen, antibodies, or detection reagents used. Blocking buffers are typically proteins or non-ionic detergents and the type used will depend on several factors, including the antibodies, antigens and detergent reagents used and the surface chemistry of the plates. The most common type of blocker, bovine serum albumin (BSA), is a protein. If your background signal is high and you suspect insufficient blocking, using a higher concentration of blocker or increasing the blocking time may help. Conversely, if you have a problem with persistent background, it might be worth investing the time to optimize the type of block you are using.[5,6]

3. Sample handling and dilution

The mishandling or incorrect storage of reagents and samples can affect downstream analysis as different buffers and reagents will have different storage requirements. For example, some must be stored in a freezer, others at room temperature and some with a light-sensitive cover (such as foil).

If there are plans to use the same samples for future experiments, then they should be placed into aliquots and taken out when needed. This will avoid multiple freeze/thaw cycles and maintain accurate results. When the samples are ready for use, they should be thawed on ice (or according to the protocol).

4. Ensuring consistency and accuracy

One way to obtain consistent and accurate results is to make sure the lab bench is appropriately set up, so that all components and instruments are within reach. Using calibrated precision pipettors will also limit any inconsistencies during the pipetting process.

Intra-assay precision is the error between wells. As mentioned previously, the inclusion of replicates is important to identify errors and anomalies. The coefficient of variability (CV) is a ratio that describes the variability within a population, independent of the absolute values of the observations.[7] When interpreting the data from an ELISA, the %CV is useful for identifying any inconsistencies in sample replicates. This is shown, post-assay, in the variation among optical density (OD) values. Overall, the lower the %CV, the more precise the results.

Inter-assay precision is the reproducibility between plates or between assays, such as those completed on different days. Although commercial ELISA kits have inter-assay quality controls, scientists must keep a detailed notebook for changes made at any point in the protocol, so that other researchers can complete the same experiment, in the same way and obtain the same results. This also enables the assay to be monitored over time to identify any systemic issues or decline in assay performance.

5. Avoiding contamination

Contamination can cause many problems in the downstream analysis of ELISA data, such as high well-to-well variation, high background values and weak or low signal intensity. There are a few ways to prevent contamination:

1. Work in a clean environment
2. Sterilize all water via deionization or distillation
3. Use new and clean reagent reservoirs for each transfer
4. Use fresh pipette tips between samples
5. Take care when preparing and applying samples to avoid splashing or cross contamination
6. If using automation, ensure the system is cleaned and reagent supplies are refreshed
7. Avoid pouring reagents back into stock bottles as this can introduce contaminants – remove only what is required

6. Proper plate washing techniques

Proper wash and rinse protocols are especially important. Insufficient, inconsistent and/or over-washing can cause problems during downstream analysis. There are many ways to wash the plates which include using an automated washer, a manual manifold or a wash bottle. When manually aspirating the fluid from the wells, be careful not to touch the bottom of the well – the tip of the pipette should be placed on the side of the well instead. To reduce the background signal, the wash volume needs to be high enough to remove unbound sample and reagents from the wells. However, over washing can reduce target signal.

Ensure all wells are washed equally to avoid introducing signal variation across the assay plate. To do this, check pipette tips are secured properly if washing manually or if using an automated plate washer, all jets that dispense and aspirate must be free from blockages. You can test them on empty plates to check that they are working equally.

7. Normalizing data

Accurate data analysis is a very important step in the ELISA workflow that addresses inter-assay variability, which can be impacted by many factors, such as temperature and development. To limit the impact of these factors on assay results, normalization should be performed by comparing the standards on each plate between experiments.

8. Staying within the dynamic range

It is vital the assay samples stay within the dynamic range of the plate reader, otherwise the results will not be representative – the dynamic range of an instrument can be found in the machine manual. When developing an assay and determining sample dilutions, both the highest and lowest positive samples should fall within the instrument's dynamic range. Standards are usually provided with ELISA kits; however, these can also be sourced independently.

Overall, the ELISA is an essential part of many laboratories. Following the tips above will help anyone produce accurate and reliable results.

For more information and to view Bethyl ELISA kits, please visit: bethyl.com/search_elisa.

References

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