Introduction to BioOcean® ELISA Product

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1. Principle of ELISA

Enzyme-linked Immunosorbent Assays (ELISAs) and Radiometric Immuno-Assay (RIA) are probably the most common assays in the lab today. Unlike ELISA, Radiometric Immuno-Assay (RIA) has similar principle but utilizes the radioactive reagents which is dangerous to users. ELISA is an antibody-based method designed to quantitatively detect a specific analyte in a sample. The analytes are often proteins, or other small molecular like vitamins. The format of these analytes can range from biological fluids (e.g. plasma, serum, urine, perspirant) to conditioned cell culture media.

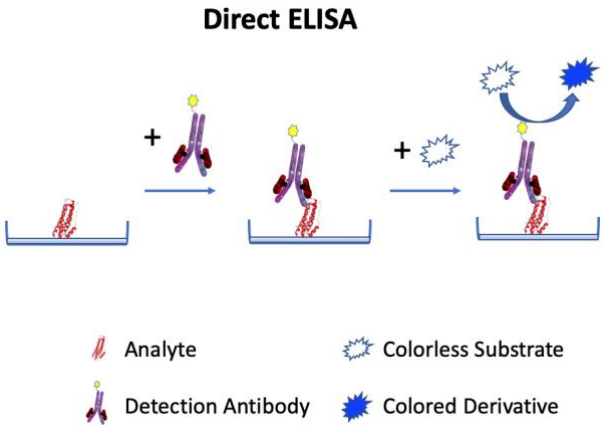
Depending on the analytes, hundreds different kinds of ELISA products are being used, and many are commercially available. The basic principle of this assay is actually quite simple. Step 1: The analyte is first captured by antibodies on solid support surfaces, commonly as 96-well microtiter plate. Step 2: The analyte is further bound by conjugated antibodies, usually to an enzyme like peroxidase. Step 3: After the excessive analytes or antibodies are washed away, a colorless chromogenic substrate is introduced. Step 4: The enzyme catalyzes the colorless substrate into a dark-colored derivative and the absorption (OD value) is recorded. In a quantitative assay the degree of colorimetric shift can be compared to the shift observed from a set of standards with known concentration, therefore to estimate the quantity of analyte

2. Choosing the right type of ELISA product

A. Direct ELISA.

The direct ELISA is the simplest ELISA format. Usually the purpose of this assay is to not quantitatively measure the analyte, but instead is to be used to qualify the reagents in the assay. It is usually a quick test before transferring the test into another accurate assay later. In a direct ELISA assay:

- The analyte is immobilized onto a 96-well microtiter plate.
- A **conjugated detection antibody** solution is added to the well. After incubation the unbound antibody is rinsed away. The specificity and affinity of the antibody are critical.
- A substrate solution like TMB (3,3',5,5'-tetramethlybenzidene) is added to the wells.
- The color change from the substrate solution is measured by a plate reader.
- The color change indicates the quantity of analyte.



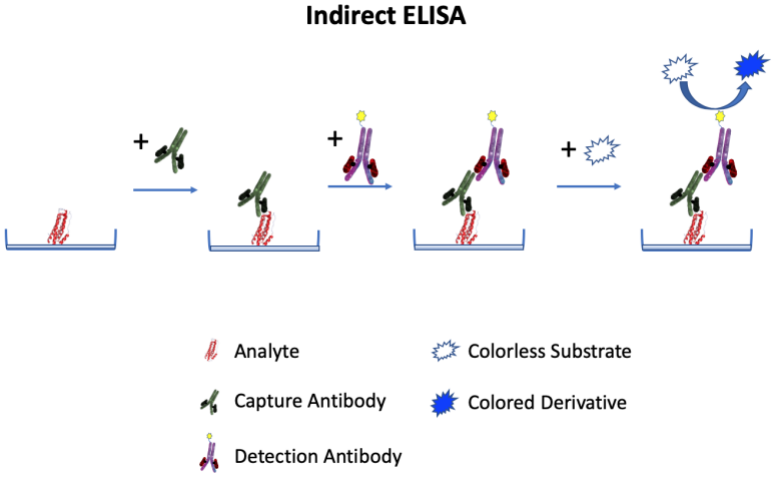
Direct ELISAs is often used to study the presence (type and concentration) of analytes compared against a set of standards with known concentration.

B. Indirect ELISA

The indirect ELISA is almost identical to direct ELISA except the primary antibody used to detect the analyte is not a conjugated antibody. Therefore, a conjugated secondary antibody must be used before adding the substrate solution**.**

In an indirect ELISA assay:

- The analyte is immobilized onto a 96-well microtiter plate.
- A **capture antibody** is added to the well and then incubated. After incubation the unbound antibody is rinsed away. The specificity and affinity of the antibody are critical.
- A **conjugated detection antibody** is added to the well and then incubated. After incubation the unbound antibody is rinsed away.
- A substrate solution like TMB (3,3',5,5'-tetramethlybenzidene) is added to the wells.
- The color change from substrate solution is measured by a plate reader.
- The color change indicates the quantity of analyte.

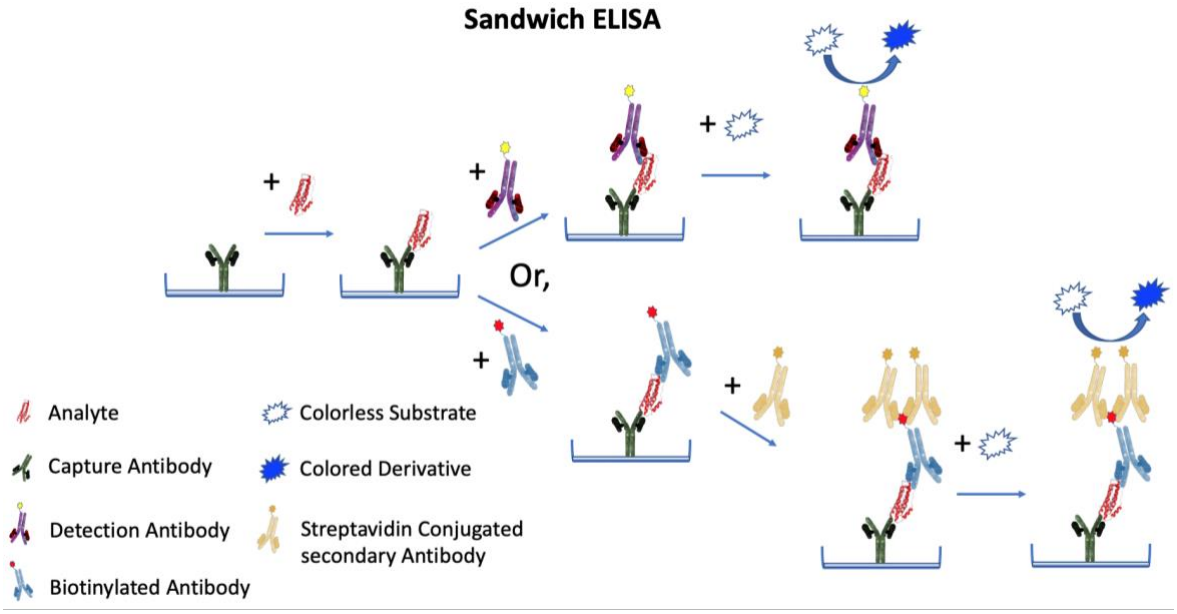


The use of a secondary antibody in an ELISA can help to amplify a weak signal, therefore a previously undetectable interaction between the analyte and antibody can be detected.

C. Sandwich ELISA

This is the most used and commercially available ELISA assay. The "sandwich" will be formed because the analyte will be retained between two or three different antibodies.
In a sandwich ELISA assay:

- A **capture antibody** is immobilized onto a 96-well microtiter plate.
- Then a solution containing the analyte is added to the well and then incubated. After incubation the unbound analyte is rinsed away.
- A **conjugated analyte-specific antibody (detection antibody)** is added to the well and incubated. After incubation the unbound antibody is rinsed away.
- If the signal needs amplification, the **conjugated analyte-specific antibody** used above is usually biotinylated, and must be followed by incubating it with Streptavidin-conjugated secondary antibody.
- A substrate solution like TMB (3,3',5,5'-tetramethlybenzidene) is then added to the wells.
- The color change from substrate solution is measured by a plate reader.
- The color change indicates the quantity of analyte.



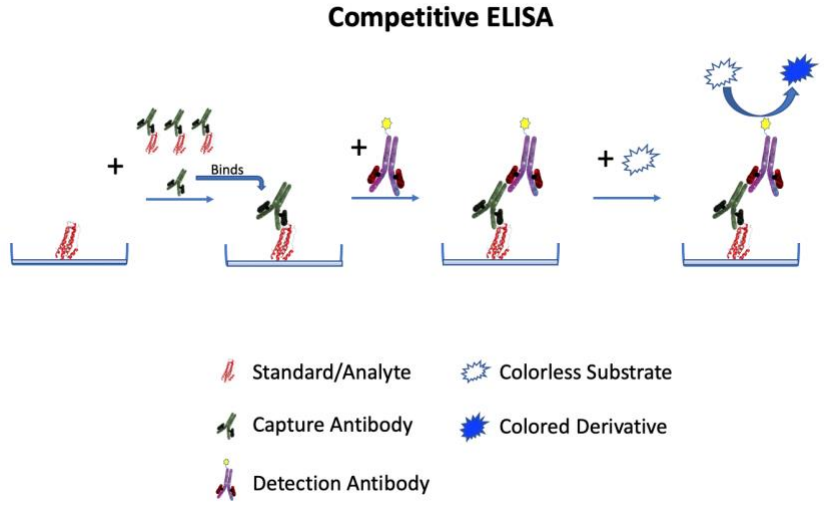
A monoclonal antibody is often to be the capture antibody in a sandwich ELISA. This helps to increase the specificity and reduce the background noise for an assay because of its high specificity.

D. Competitive ELISA

The competitive format ELISA is the most difficult assay among all ELISA tests.
In a Competitive ELISA:

- Standard was immobilized onto a 96-well microtiter plate first. Standard should be the same as the analyte in term of being detected by the antigen-specific detection antibody.

• Incubate the detection antibody with the analyte in a tube. Then add the mix to 96-well microtiter plate.
• Only the unoccupied detection antibody can bind to the immobilized standard in the well.
• A substrate solution like TMB (3,3',5,5'-tetramethlybenzidene) is added to the wells.
• The color change from substrate solution is measured by a plate reader.
• The color change indicates the quantity of analyte. In a competitive ELISA, a higher OD value indicates a lower concentration of the analyte.



3. Tips to users

The basic requirement for running ELISA
In order for a sample to be tested by ELISA, the sample should meet at least the following:
The analyte can be recognized by an antibody within detectable ranges.
The analyte must be in a solution from 50-200µL with or without dilution.
The sample must NOT contain any substance that will interfere with antibody recognition.
The sample must NOT interfere with colorimetric development.

Average time to run an ELISA assay
As a fast, simple, and accurate test, the ELISA assay usually completes within 4-8 hours.

Average number of tests in an ELISA assay
A single 96-test kit is generally capable of testing 40 samples in duplicate as well as an 8-sample standard curve.

High sensitivity ELISA kit
Depending on the requirement, some kits that offer higher sensitivity compared to the regular kits should be chosen by user. The low detection limits are usually 10-100 times lower than the regular kit. However, the upper limits are usually much lower than regular kit. Users may test both kits at the same time and compare before performing more assays.

High-throughput capability
ELISA not only does quick assays, but it also can be easily adapted to run a few hundreds of samples in a day.

Interfering substance:
The following substances should be considered:
Some samples that contain certain detergents, or strong acids/ strong bases.
Some samples that may have absorption and significant interference as the assay-developed.

ELISA assay to distinguish isoforms
The short answer is possibly yes. If an assay using a pair of antibodies that can distinguish between different isoforms of the same protein in a sample, then ELISA assay is able to detect that specific isoform. User needs to understand this limitation and apply other techniques to distinguish these isoforms if needed.

Pre-made ELISA vs. DIY kit
Users might be interested in DIY ELISA kit because it is usually cheaper. However the drawback to this is, that users should keep in mind that a premade ELISA kit usually has better performance, consistence and quality control because of the following reasons listed below:

Saves time: Optimization of an ELISA assay is very time consuming. Users should consider the value of their time that could be used to analyze, and even to publish result.

Compatibility: A pre-made ELISA kit contains all reagents that are tested and show compatibility with each other. Users often encountered incompatible reagents during DIY that should have be avoided in the pre-made ELISA kits.

Quality Control: From time to time, the components used for ELISA assay may have changed and to be able to quality their use, users must spend more time to investigate and titrate all conditions again. With a pre-made kit that has been tested and meet the quality standards, users can feel more confident and convenient to start the assay without hesitation.

Cost-Effective: There are hundreds of ELISA kits that are low cost and high performance. Bio-Ocean offers 100% satisfaction guarantee for all BioOcean® ELISA kits. The technical support team is also ready to help customers with any technical issue or even to performance troubleshooting for customers.

4. Summary

ELISA is more powerful assay than many other proteomics techniques. We hope you now have better idea of what you need and what Bio-Ocean can offer to you. We offer premium quality ELISA kits that can meet your expectation, with a 100% satisfaction guarantee. Our scientists are ready to answer any of your questions. Feel free to contact us if you have any question.