ELISA Protocol

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Introduction

ELISA (Enzyme-Linked Immunosorbent Assay) is a sensitive biochemical technique for detecting and quantifying proteins, antibodies, hormones, and other biomolecules in biological samples.

Various types of ELISA exist, including direct, indirect, sandwich, and competitive ELISA procedures. Sandwich ELISA is particularly beneficial for detecting low-abundance proteins in complex samples, as it employs two antibodies for improved specificity and sensitivity.

Reliable results depend heavily on protocol adherence, quality reagents, and optimized conditions. Boston BioProducts offers high-quality reagents specifically designed for consistent, accurate ELISA outcomes.

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Want to keep our ELISA protocol on hand for quick reference in the lab?

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Further Reading

A small-molecule inhibitor of C5 complement protein (Jendza et al., 2019)

Angiostatin regulates the expression of antiangiogenic and proapoptotic pathways via targeted inhibition of mitochondrial proteins (Lee et al., 2009).

Materials & Reagents

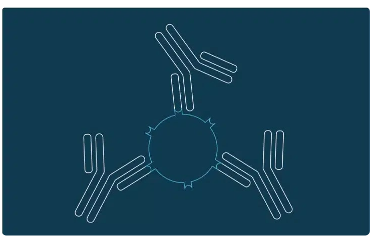
Here's everything you need to carry out our ELISA protocol. In addition to Boston BioProducts' high-quality buffers and reagents, you'll need the following materials and equipment:

- 96-well microplate
- Capture and detection antibodies
- Plate reader (spectrophotometer)
- Multichannel pipettes and tips
- Plate sealer films

Reagent	Product Name	Link
Coating Buffer	Borate Buffer (0.5M, pH 8.5) Carbonate-Bicarbonate Buffer (1M, pH 9.0)	View Product View Product
Blocking Buffer	Casein (3%, PBS) Bovine Serum Albumin (BSA, 5% TBST) Gelatin (10%) Casein (3%, TBS) Non-Fat Powdered Milk	View Product View Product View Product View Product View Product
Wash Buffer	PBST (10X, 0.5% Tween, pH 7.4) PBST (20X, 2% Tween, pH 7.4) TBST (20X, 2% Tween, pH 7.4) TBST (10X, 0.5% Tween, pH 7.6) PBS (10X, pH 7.4) Citric Acid Buffer (2M)	View Product View Product View Product View Product View Product View Product
Antibody Dilution Buffer	PBST (0.05% Tween-20, pH 7.4) TBST (0.05% Tween-20, pH 7.4) Bovine Serum Albumin (BSA, 5% TBST) Tris-HCl Buffer (1M, pH 8.0)	View Product View Product View Product View Product
Stop Solution	Sodium Hydroxide (NaOH, 1N) Hydrochloric Acid (HCL, 1N) 10% SDS 20% SDS EDTA (0.5M, pH 8.0, RNase-free) Acetic Acid (2M)	View Product View Product View Product View Product View Product View Product

Boston BioProducts' Protocol

This ELISA protocol outlines key steps, from plate coating to final signal detection, for a reproducible and optimized ELISA workflow. While this protocol primarily details the Sandwich ELISA workflow, it also provides insights into the other ELISA types to help you select the best assay for your research needs.



Sample Preparation

Your sample preparation may vary, but optimizing this step is a critical part of the ELISA protocol. It ensures antigen integrity and reduces background interference, leading to better results.

Our general advice by sample type:

- **Serum or plasma:** Centrifuge to remove debris and dilute in blocking buffer or sample diluent to fall within the assay's dynamic range.
- **Cell culture supernatant:** Clarify by centrifugation and store on ice or at -20°C until use.
- **Tissue or cell lysates:** Use a lysis buffer compatible with ELISA (avoid detergents or agents that interfere with antibody binding). Centrifuge lysates and use the supernatant.

> Avoid repeated freeze-thaw cycles, which can degrade protein targets.

Coating

Coating the plate with the capture antibody enables specific immobilization of the target antigen, forming the foundation for sensitive and selective detection in Sandwich ELISA. If you're using a kit with pre-coated plates, you can proceed directly to the blocking step.

1. Dilute your capture antibody in Carbonate-Bicarbonate Buffer or Borate Buffer.
2. Add 100 µl to each well, seal the plate, and incubate overnight at 4°C.

   > To preserve coated antibodies during longer storage, consider adding a protein stabilizer such as trehalose or sucrose.
3. Remove excess solution and wash 3x with wash buffer.

Blocking

Blocking prevents non-specific binding by saturating uncoated areas of the well, helping to reduce background noise and improve assay sensitivity.

1. Add 200 µl of a blocking solution to each well.

   > Other recommended blocking agents include Casein (3% in PBS or TBS), Gelatin (10%), and milk powder, depending on compatibility with your assay system.
2. Incubate for 1 hour at room temperature.
3. Aspirate and wash 3x with wash buffer.

Sample Addition

During this step, the antigen from your sample binds specifically to the immobilized capture antibody. Accurate sample dilution and incubation are essential to ensure optimal binding and reliable quantification within the assay's dynamic range.

Not doing a sandwich ELISA?

Here's how direct, indirect, and competitive ELISA procedures differ.

Mini direct ELISA protocol

1. Coat the plate directly with antigen.
2. Block with BSA, Casein, or milk powder.
3. Add enzyme-conjugated primary antibody.
4. Incubate, wash, and add TMB substrate.
5. Stop the reaction and read at 450 nm.

   > ⚠ Direct ELISA has fewer steps but lower signal amplification and higher background risk, making it a simple and fast ELISA protocol ideal for detecting a strong, high-purity antigen.

Mini indirect ELISA protocol

1. Coat plate with known antigen.
2. Block with Casein or BSA in TBST.
3. Add a sample containing primary antibody.
4. Add enzyme-linked secondary antibody.
5. Develop with the substrate, stop, and read the plate.

   > The indirect ELISA protocol provides signal amplification but may introduce cross-reactivity with the secondary antibody. It is useful for detecting antibodies in a sample, such as in serological testing.

Mini competitive ELISA protocol

1. Coat plate with known antigen.
2. Incubate sample antigen with enzyme-conjugated antibody.
3. Add mixture to the coated well.
4. Wash, develop signal, stop reaction, and read.

   > ⚠ Signal intensity is inversely proportional to sample concentration in competitive ELISA, therefore the competitive ELISA procedure is mostly used when the analyte is small or available in limited quantities.

1. Prepare antigen standards and samples in a compatible diluent, such as PBST or TBST.
2. Add 100 µl per well and incubate 1–2 hours at room temperature.
3. Wash thoroughly 3x with wash buffer.

Detection Antibody Incubation

The detection antibody binds to a second epitope on the captured antigen, forming the "sandwich" complex. When enzyme-conjugated, this antibody enables signal generation upon substrate addition, allowing quantification of your target analyte.

1. Dilute your enzyme-conjugated detection antibody in the same antibody diluent used in the previous step.
2. Add 100 µl per well and incubate 1 hour at room temperature.
3. Wash 5x with a stringent wash buffer to remove unbound antibodies.

Substrate Incubation

The substrate reacts with the enzyme linked to the detection antibody to produce a measurable signal—typically a color change. Therefore, the enzyme used dictates which kind of substrate to choose.

1. Add 100 μl of substrate to each well—including blanks—and incubate the plate at room temperature, protected from light.

   > If using horseradish peroxidase (HRP)-conjugated antibodies, add TMB substrate (preferably cold) and incubate for 15 minutes.

   > If using alkaline phosphatase (ALP)-conjugated antibodies, use pNPP substrate and incubate for 30-60 minutes.

Stop Reaction

Stopping the enzymatic reaction preserves the developed signal and prevents further color change. The choice of stop solution depends on the enzyme used and the substrate system, with acidic or denaturing agents commonly employed to halt activity.

1. Add 50 μl of stop solution to each well.

   > If using a TMB reaction, add Sulfuric Acid. The solution will change from blue to yellow.

   > For ALP-based assays or alternative substrates, other stop solutions such as Sodium Hydroxide, Hydrochloric Acid, EDTA, or Acetic Acid may be appropriate.

   > For specific applications, SDS may be used to denature the enzyme and stop the reaction without affecting pH-sensitive signals.

2. Immediately measure absorbance at the appropriate wavelength for your substrate using a plate reader (e.g., typically 450 nm for TMB, 405 nm for pNPP).

   > Incubation time should be optimized to achieve sufficient signal intensity without exceeding the linear range of detection.

Data Analysis

Once the plate has been read, ELISA results are analyzed by comparing sample absorbance values to a standard curve. Accurate curve fitting and replicate consistency are essential for quantifying analyte concentrations with confidence.

1. Subtract the average blank absorbance (e.g., wells with buffer only) from all sample and standard values to correct for background.
2. Plot the standard curve using known antigen concentrations (typically log concentration vs. absorbance).
3. Apply curve fitting—commonly linear regression or a 4-parameter logistic (4PL) model—depending on your assay's range and shape.
4. Use the curve to interpolate unknown sample concentrations from their absorbance values.
5. Check replicates for consistency and exclude any outliers that deviate significantly.

   > For best results, ensure your sample values fall within the linear portion of the standard curve and re-run samples that fall outside this range.

Troubleshooting & Optimization Tips for ELISA Protocols

My background signal is too high

The problem:

High background can result from non-specific binding, insufficient washing, or suboptimal blocking.

What to do:

- Increase wash stringency (more washes, higher % Tween-20))
- Try a different blocking agent (e.g., BSA vs. casein)
- Ensure incubation conditions aren't too long or warm

My signals are weak or inconsistent

The problem:

Inadequate antigen capture or low antibody affinity may reduce signal intensity.

What to do:

- Confirm antibody concentrations and storage conditions
- Increase sample incubation time
- Ensure the antigen or sample wasn't degraded during prep

My replicates are highly variable

The problem:

Pipetting error, evaporation, or inconsistent plate handling can affect reproducibility.

What to do:

- Use a multichannel pipette
- Pre-wet tips
- Seal plates during incubation
- Avoid edge effects by not analyzing outer wells unless blocked and filled properly

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Why Boston BioProducts?

Boston BioProducts offers a comprehensive portfolio of high-performance reagents and buffers specifically optimized for ELISA workflows. From coating and blocking to washing and signal development, our solutions are formulated to enhance specificity, reduce background, and support consistent, reproducible data.

Whether you're developing a custom assay or using a commercial kit, our buffers help you get the most out of your ELISA protocol—saving time, minimizing troubleshooting, and ensuring reliable quantitative results every time.

FAQs

− What is the difference between Sandwich and Direct ELISA protocols?

The key difference lies in antibody usage and assay design. Sandwich ELISA uses two antibodies—a capture antibody immobilized on the plate and a detection antibody that binds to a different epitope on the same antigen. This format offers high specificity and sensitivity, making it ideal for detecting low-abundance targets in complex samples like serum or cell lysates.

In contrast, a Direct ELISA protocol uses a single enzyme-conjugated primary antibody that binds directly to the immobilized antigen. This approach is faster and simpler, with fewer steps and reagents, but it typically results in lower sensitivity and higher background, especially in unpurified or complex sample matrices.

+ Can I reuse ELISA plates?

+ What's the best way to choose a blocking buffer?

Have A Question?

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