

ELISA Kit Troubleshooting Tips:

4 Common Challenges And How To Resolve Them





Introduction

This document provides a comprehensive guide to troubleshooting common challenges encountered when performing Enzyme-Linked Immunosorbent Assays (ELISAs) in a scientific laboratory setting. It discusses four main challenges:

- 1) Poor Standard Curve
- 2) No Signal
- 3) High Coefficient of Variance (CV), and
- 4) High Background



Throughout the document, practical tips and recommendations are provided to help scientists and researchers overcome these challenges and ensure the accuracy and reliability of their ELISA assays.

About ARP American Research Products

Based in Massachusetts, ARP has been providing high-quality products to research laboratories in hospitals, universities, and biotech firms worldwide since 1994.

We offer Monoclonal and Polyclonal Antibodies as well as purified and recombinant Antigens for research. Our catalog of life science tools is comprised of products for the detection of Cytokines and Growth Factors, Infectious/TORCH and Autoimmune diseases, Bone and Mineral Metabolism, Cardiac Markers, Catecholamines and Bioamines, Reproductive and Fertility Assays, Tumor Markers, Steroids, as well as Endocrinology, Neurology and Salivary Assays.

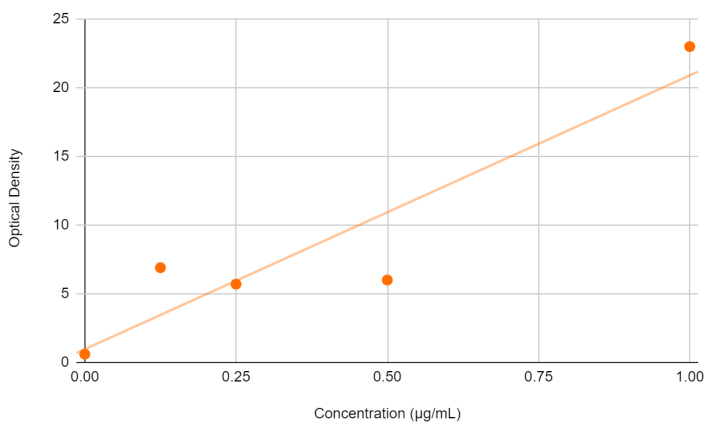


The ARP Promise:

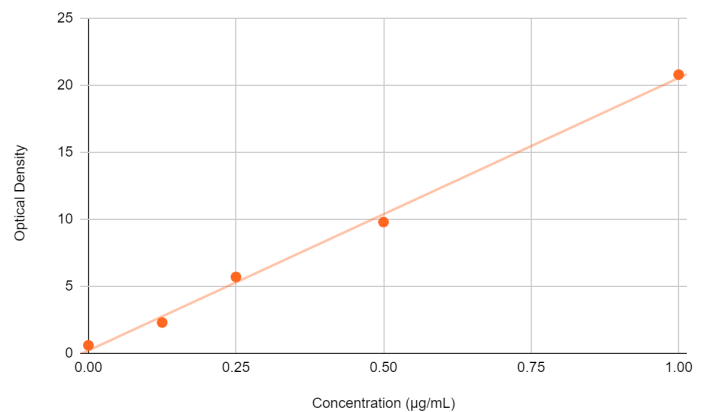
Our commitment to detail and quality will ensure the results you expect. We are continuously adding new products to our database to accommodate your changing research needs.

Challenge 1: Poor Standard Curve

Let's explore the possible causes and solutions for obtaining a poor standard curve in your ELISA. The standard curve is crucial as it allows you to determine the concentration of the target molecule in your sample. It may not always be apparent if your standard curve is incorrect, so building confidence in it is the initial step. We strongly recommend using or preparing quality control (QC) samples for each ELISA you perform. Ideally, these should span low, medium, and high concentrations, covering the entire range of the standard curve. Place them at both the left and right-hand sides of the ELISA plate (Columns 1 and 12). These QC samples should have different concentrations to those on your standard curve, simulating actual samples with known concentrations for accuracy validation. Ideally, match the QC sample matrix with your real sample, such as serum or plasma. It's also advisable to prepare a batch of QC samples in advance and store them at -80°C, like your regular samples. This enables you to monitor assay performance over time or across different manufacturing lots, assuming your analyte remains stable under your storage conditions. If you anticipate needing new QC samples in the future, prepare them before exhausting the older batch so you can run both old and new lots in parallel on a test assay to ensure equivalency.



Example of poor standard curve



Example of good standard curve

Common Issues with the Standard Curve

Common issues with the standard curve may include inconsistent optical density (OD) values at different concentrations, resulting in a poorly fitting curve with a low R² value (ideally, R² should be >0.99).

Incorrect Standard Solution: Using the wrong concentration of your starting stock for a standard may result in standards that are either under- or over-diluted, leading to deviations in OD and shifting your standard curve out of range.

Solution: Double-check the standard stock concentration, dilution calculations, and your dilutions. If you're reconstituting a standard from a lyophilized vial, ensure you follow the manufacturer's instructions. It's advisable to vortex vials to ensure complete reconstitution.

Degraded Standard: An old or improperly stored standard may have degraded, resulting in lower OD values than a non-degraded standard.

Solution: Verify that the standard has been reconstituted correctly and stored appropriately.

Curve Doesn't Fit the Scale: Sometimes, you may observe higher or lower OD values than usual, even when you believe the curve was prepared correctly. This can be attributed to variations in absorbance due to factors like different batches of detection reagents.

Solution: When using a specific manufacturer's ELISA kit for the first time, ensure you use the recommended curve fitting model, typically a 4- or 5-parameter logistic curve fitting model (4-PL or 5-PL). If necessary, try other curve-fitting models like log-log. Consistency is key, so changing the curve fit model may render your results incomparable between assays, especially if absolute values are required.

Pipetting Error: Pipetting errors, such as incorrect sample volumes or high coefficients of variation (CVs), can occur when adding samples to the plate.

Solution: To minimize pipetting errors, ensure that the pipette tip is securely attached, change tips between reagents and samples, eliminate air bubbles in the well or pipette tip, dispense reagent/sample against the well's side, and rinse tips with successive aspiration/dispense cycles.

***General Tip:** Keep your tubes until you have analyzed your results. Maintaining tubes used for preparing standards, QC samples, and samples allows you to double-check for dilution errors or tube mix-ups when you spot an error later.*

Challenge 2: No Signal

Let's explore the causes and potential solutions for the absence or very weak signal in your ELISA results. Since every step in an ELISA is critical, there are numerous potential causes for a lack of signal, making troubleshooting a comprehensive process.

No Sample Signal, but Standards and/or QC Samples Are Fine

If your standard/calibration curve and/or QC samples show a signal but your samples do not (when expected), there may be issues with your samples. Several possible problems could arise:

- **Using a New Sample Matrix:** Not all ELISAs work with all sample types, and switching to a different sample type may require consultation with the manufacturer or assay redevelopment.
- **Dilutions Too High:** Over-dilution of samples beyond the ELISA's sensitivity could result in signal loss.
- **Analyte Below Detection Limit:** Samples may contain insufficient analyte, falling below the ELISA's detection limit.
- **Degraded sample**

No Signal at All

Several potential causes can lead to zero signal development:

Incubation Times/Temperature: Verify that incubation times and temperatures align with the manufacturer's guidelines to avoid affecting assay kinetics.

Insufficient/Incorrect Capture/Detection Antibody: Double-check antibody dilutions, especially in-house prepared reagents, and ensure correct order usage for ready-to-use reagents from the manufacturer.

Not Enough Capture/Detection Reagent Added: Ensure correct volumes of reagents were added to the plate.

Reader Wavelength: Confirm the correct wavelength settings on your plate reader, particularly when using substrates requiring visual stopping.

Substrate Solution: Use the correct substrate and check stock solution expiry dates.

Plate Washing: Avoid over-vigorous or extended plate washing that may wash away analyte or reagents.

Dry Wells: Prevent wells from drying out to avoid unpredictable ELISA issues.

Challenge 3: High CV

Let's delve into the possible causes of high coefficient of variance (CV) in your ELISA results. High CV can manifest as significantly different optical density readings between duplicate wells and may affect certain parts of the plate differently. Addressing the problem depends on identifying the specific location of high CV, as it can impact curve fitting and result reliability.

Here are some common causes of high CV in ELISAs:

- **Pipetting Error:** Although often attributed to pipetting errors, high CV can also result from errors in sample preparation, reagent handling, and washing steps. Ensure proper mixing of diluted samples to avoid differences between duplicates.
- **Sample Preparation:** Vigorously mix or pipette diluted samples before adding them to the plate to avoid inconsistent analyte distribution.
- **Reagent Preparation:** Poor mixing of reagents, such as capture or detection antibodies, may lead to varying reagent levels in different wells, increasing CV.
- **Plate Washing:** Ensure thorough and consistent plate washing to prevent leftover matrix components on the plate, contributing to increased background signal.
- **Bubbles in Wells:** Minimize bubbles during pipetting, as they can interfere with analyte or reagents. Pop any visible bubbles before reading the plate.
- **Edge Effects:** Pay attention to edge effects, which can result from temperature differences across the plate. Control temperature and ensure proper reagent mixing to mitigate this issue.

Challenge 4: High Background

High background in an ELISA manifests as excessively elevated color development or optical density readings across the entire plate. High background decreases the signal-to-noise ratio, reducing assay sensitivity and potentially rendering results unusable. There tends to be two main reasons for high background: plate washing and plate blocking. However, we'll delve into a little more detail below.

General tip: *If you're running a lot of the same ELISA over time, tabulate the optical densities for the blank (negative control), standards, and any QC sample wells. This can give you an idea if the assay or a reagent is changing over time, leading to a gradual shift in the kinetics of your assay.*

Antibody-Based Problems:

- **Coating/Detection Antibody Concentration:** Double-check the dilution of the capture antibody, particularly if you're developing the ELISA from scratch. Optimization of coating conditions may be necessary in such cases.
- **Non-Specific Binding:** Review the formulation of your antibodies and ensure that the diluent used is suitable, as incompatible diluents can lead to non-specific binding. Adequate plate blocking is crucial in reducing non-specific binding.
- **Cross Reactivity:** When switching to new lots, suppliers, or types of antibodies, check for cross-reactivity with other analytes in your sample type using a negative control.

Reagent (Buffer or Substrate) Issues:

- **Contamination:** When repeating an assay due to previous high background, use fresh reagents throughout. Contaminated buffers can lead to high background.

Matrix Effects:

- The term 'matrix effect' refers to signal interference generated by non-target sample matrix components. It is assigned to the increase in signal observed by the sum of 'all' sample composition components on the final assay reading. Certain matrices have a greater impact on the final assay signal than others. Consult the manufacturer for information on validated sample types for kit-based assays, or optimize your assay for new sample types if you've developed it yourself.
- A Spike and recovery experiments will allow you to assess the impact of the sample matrix on your specific assay, which is particularly important when developing an ELISA from component reagents as opposed to the use of a pre-formatted kit. Add a known amount of analyte to both the sample matrix and the diluent used to prepare the standard. The ELISA should then be run to completion with both sample types and the response calculated against the standard curve allowing you to ascertain the matrix effect within your assay.
- If a significant matrix effect is observed, dilute the standard in a matrix more closely formulated to that of the sample (e.g. cell lysis buffer, or serum).

Plate Blocking:

The blocking buffer is crucial in an assay as it prevents non-specific binding. To reduce high background, consider increasing the blocking solution's concentration or adding a small amount of a non-ionic detergent, such as Tween-20. Extending the blocking step incubation time and using a plate shaker can also help.

Plate Washing:

- Inadequate plate washing can leave matrix components on the plate, leading to increased background signal. Additional wash steps or brief incubations between wash steps can be effective in reducing background. Ensure automated plate washers are functioning correctly and that tubing is clean and well-rinsed between changes of wash buffers.

Substrate Issues:

- Some substrates, like TMB, can precipitate if the reaction is not stopped within a specified time. Follow the manufacturer's recommendations and read the plate promptly after addition of stop solution. Ensure the bottom of your assay plate is clean to prevent interference with the plate reader.

ARP offers a wide range of ELISA kits for scientific research. Search ARP1.com to find the right ELISA kit for your next project, or connect with us at info@arp1.com.

