# ELISA Troubleshooting Guide

Before using a Beacon Enzyme-Linked Immunosorbent Assay (ELISA) Kit, carefully review the kit brochure. Assay timing, volumes, and protocols can vary, and deviations may lead to inaccurate results. For best results, ensure consistency, precision, and a clean laboratory environment. If you encounter issues, use this troubleshooting guide to help resolve them quickly.

| **Issue: Inconsistent results or high variability** | |
|---|---|
| **Possible Cause** | **Solution** |
| Pipetting errors | Use calibrated pipettes that are capable of delivering the intended volumes, and ensure they are periodically calibrated according to your lab's quality assurance schedule or the manufacturer's guidelines. Choose the correct tip size and type, and replace tips when switching between incompatible reagents. For plate assays, use a multichannel pipette; for tube assays, use a repeater pipette as specified in the product brochure. When using a multichannel pipette, ensure all tips are securely in place and reagent levels are even. Maintain a consistent pipetting technique and always run calibrators, controls, and samples in replicates. Use a positive displacement pipette for calibrators, controls, and samples containing more than 10% methanol. |
| Optical surface damage or debris in wells/tubes | Gently wipe the optical surface with a lint-free wipe. Tap inverted wells/tubes on an absorbent material (e.g. paper towel) to remove debris. |
| Contaminated work area | Keep the work area clean to prevent contamination. |
| Reuse of wells/tubes | Always use fresh wells/tubes for each assay. Do not reuse wells/tubes. |
| Low zero calibrator Optical Density (OD) (<0.6) | See the section below on low OD. |
| **Issue: Low Optical Density (OD) of the zero calibrator** | |
| **Possible Cause** | **Solution** |
| Kit components not at room temperature | Allow all kit components to reach room temperature before use. |
| Improper storage of kits | Follow the storage guidelines in the product brochure. |

| | |
|---|---|
| Incorrect incubation time | Use a timer to track incubation times precisely. |
| Insufficient reagent mixing | Gently shake plates/tubes for at least 30 seconds to ensure thorough mixing. Be careful to avoid spills. |
| Expired or mixed lots | Check expiration dates and do not mix kit components from different kit lots. |
| **Issue: High background** | |
| **Possible Cause** | **Solution** |
| Incorrect wash solution used | Use the correct wash solution specified in the brochure. |
| Incomplete or incorrect washing | Completely flood wells/tubes with wash solution. Decant immediately, avoid soaking. Be sure to decant in between washes. Be sure to tap inverted wells/tubes on an absorbent material (e.g. paper towel) after the final wash to remove excess wash solution. |
| **Issue: Out-of-range or unexpected sample results** | |
| **Possible Cause** | **Solution** |
| Incorrect sample extraction protocol | Follow the exact sample extraction protocol outlined in the brochure. |
| Mismatched calibrator diluent | Ensure final sample extracts match the calibrator diluent (this should not be a problem if following the protocol detailed in the product brochure). |
| Cross-reactivity | Check the kit brochure for known cross-reactivity. |
| Sample concentration too high | If sample readings exceed the highest calibrator value, dilute the sample before testing. |
| Incorrect calculations | Double-check all calculations, including dilution of calibrators, controls, and samples. |

For further technical support, contact us!

**Phone Number:** +1-207-571-4302
**Technical Support Email:** info@beaconkits.com