ELISA FAQs

Discover practical solutions for your ELISA experiments with this useful troubleshooting guide.

1. Poor standard curve

Improper standard solution

Confirm dilutions are made correctly.

Standard improperly reconstituted

Briefly spin vial before opening; inspect for undissolved material after reconstituting.

Standard degraded

Store and handle standard as recommended.

Curve doesn't fit scale

Try plotting using different scales e.g. log-log, 5 parameter logistic curve fit.

Pipetting error

Use calibrated pipettes and proper pipetting technique.

2. No signal

Incubation time too short

Incubate samples overnight at 4°C or follow the manufacturer guidelines.

Target present below detection limits of assay

Decrease dilution factor or concentrate samples.

Incompatible sample type

Detection may be reduced or absent in untested sample types. Include a sample that the assay is known to detect a positive control.

Recognition of epitope impeded by adsorption to plate

To enhance detection of a peptide by direct or indirect ELISA, conjugate peptide to a large carrier protein before coating onto the microtiter plate.

Assay buffer compatibility

Ensure assay buffer is compatible with target of interest (e.g. enzymatic activity retained, protein interactions retained).

Not enough detection reagent

Increase concentration or amount of detection reagent, following manufacturer guidelines.

Sample prepared incorrectly

Ensure proper sample preparation/dilution. Samples may be incompatible with microtiter plate assay format.

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Insufficient antibody

Try different concentrations/dilutions of antibody.

Incubation temperature too low

Ensure the incubations are carried out at the correct temperature. All reagents including plate should be at room temperature or as recommended by the manufacturer before proceeding.

Incorrect wavelength

Verify the wavelength and read plate again.

Plate washings too vigorous

Check and ensure correct pressure in automatic wash system. Pipette wash buffer gently if washes are done manually.

Wells dried out

Do not allow wells to become dry once the assay has started. Cover the plate using sealing film or tape for all incubations.

Slow color development of enzymatic reaction

Prepare substrate solution immediately before use. Ensure the stock solution has not expired and is not contaminated. Allow longer incubation.

3. Large coefficient of variation (CV)

Bubbles in wells

Ensure no bubbles are present prior to reading plate.

Wells not washed equally/thoroughly

Check that all ports of the plate washer are unobstructed. Wash wells as recommended.

Incomplete reagent mixing

Ensure all reagents are mixed thoroughly.

Inconsistent pipetting

Use calibrated pipettes and proper technique to ensure accurate pipetting.

Edge effects

Ensure the plate and all reagents are at room temperature.

Inconsistent sample preparation or storage

Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaw cycles).

4. High background

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Wells are insufficiently washed

Wash wells as per protocol recommendations.

Contaminated wash buffer

Prepare fresh water buffer.

Too much detection reagent

Ensure the reagent has been diluted properly or decrease the recommended concentration of detection reagent.

Blocking buffer ineffective (e.g. detection reagent binds blocker; wells not completely blocked)

Try different blocking reagent and/or add blocking reagent to wash buffer.

Salt concentration of incubation/wash buffers

Increasing salt concentrations may reduce non-specific and/or weak off-target interactions.

Waiting too long to read plate after adding stop solution

Read plate immediately after adding stop solution.

Non-specific binding of antibody

Use suitable blocking buffers e.g. BSA or 5-10% normal serum - species same as primary antibody if using a directly conjugated detection antibody or same as secondary if using conjugated secondary. Ensure wells are pre-processed to prevent non-specific attachment.

High antibody concentration

Try different dilutions for optimal results.

Substrate incubation carried out in light

Substrate incubations should be carried out in the dark or as recommended by manufacturer.

Precipitate formed in wells upon substrate addition

Increase dilution factor of sample or decrease concentration of substrate.

Dirty plate

Clean the plate bottom.

5. Low sensitivity

Improper storage of ELISA kit

Store all reagents as recommended. Please note that all reagents may not have identical storage requirements.

Not enough target

Concentrate sample or reduce sample dilution.

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Inactive detection reagent

Ensure reporter enzyme/fluor has the expected activity.

Plate reader settings incorrect

Ensure plate reader is set to read the correct absorbance wavelength or excitation/emission wavelengths for fluorescent detection.

Assay format not sensitive enough

Switch to a more sensitive detection system (e.g. colorimeteric to chemiluminescence / fluorescence). Switch to a more sensitive assay type (e.g. direct ELISA to sandwich ELISA). Lengthen incubation times or increase temperature.

Target poorly adsorbs to microtiter plate

Covalently link target to microtiter plate.

Not enough substrate

Add more substrate.

Incompatible sample type (e.g. serum vs. cell extract)

Detection may be reduced or absent in untested sample types. Include a sample that the assay is known to detect as a positive control.

Interfering buffers or sample ingredients

Check reagents for any interfering chemicals. For example, sodium azide in antibodies inhibit HRP enzyme and EDTA used as anticoagulent for plasma collection inhibits enzymatic reactions.

Mixing or substituting reagents from different kits

Avoid mixing components from different kits.

ELISA quantification of plasma and serum occasionally encounters problems which are caused by the matrix effect. The matrix effect can arise from a number of matrix components including, but not limited to: interaction between endogenous biological components such as phospholipids, carbohydrates and endogenous metabolites (bilirubin) or an interaction between the analyte of interest and the matrix, such as covalent binding to plasma proteins. This results in erroneous sample readings.

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