

ELISA Guide; Part 4: Troubleshooting | 1

Overview

- No Signal or Weak Signal
- High Background
- Poor Reproducibility Between Plates
- Poor Reproducibility Between Runs
- Edge Effects / Plate Drift

ELISA Troubleshooting

Several common issues can occur when running an ELISA. These are detailed in the following table, along with suggested solutions.

No signal or weak signal

Capture antigen or antibody may not have adhered to the microplate

Check the binding capacity of the microplate according to the manufacturer's description

Try using a different coating buffer

Increase the incubation time for plate coating

Increase the concentration of the capture antibody and/or the analyte-specific detection antibody

Concentration of analyte-specific

Increase the incubation time for analyte-specific antibody binding

Analyte-specific detection antibody and

secondary antibody may be incompatible

antibodies may be too low

Confirm that the host species of the analyte-specific detection antibody is compatible with the secondary antibody (for example, if the analyte-specific detection antibody was raised in rabbit, an anti-rabbit secondary antibody is required for detection)

Confirm that the capture and detection antibodies recognize different epitopes

Capture and detection antibodies may be competing for the same epitope (sandwich ELISA)

Switch to using a matched antibody pair if available Consider using a different ELISA format (e.g. try using an indirect ELISA that requires only one analyte-specific antibody rather than a sandwich ELISA)

Fluorophore-conjugated antibodies may have been compromised by exposure to light

Ensure fluorophore-conjugated antibodies are stored correctly

If only the standard wells (and not the

Protect fluorophore-conjugated antibodies from light when adding them to, and incubating them in, the microplate wells Try using a fresh vial of standard

sample wells) are affected, the standard may have degraded

Check that the standard has been prepared and stored correctly

Azide (often added to antibody storage buffers as a preservative) may be inhibiting HRP activity

Ensure sufficient washing to remove any residual traces of

azide



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No signal or weak signal

Sample material may contain only low levels of the target analyte

Obtain more concentrated samples

Spike samples with a known amount of analyte to check the

sample matrix is not a source of interference

ELISA kits / kit components may have been

stored incorrectly

Check the manufacturer's instructions for storage

Plate may have been read at an incorrect

wavelength

Check the reader settings are compatible with the chosen

detection method

High Background

Increase the number and/or duration of wash steps

buffers

Try using a more concentrated blocking solution

Switch to using a different blocking buffer

Decrease the concentration of the capture antibody, analyte-

Antibody concentration(s) may be too high specific detection antibody, or secondary antibody

Decrease the antibody incubation time

Colorimetric substrates may have been

prepared too early

Always prepare substrates such as TMB immediately prior to

use to avoid unwanted color development

Microplates may have sat around after the

addition of stop solution (colorimetric detection)

Read colorimetric assays as soon as the stop solution has

been added

Consumables such as pipette tips,

reservoirs or buffers may have introduced

contamination

Use fresh plasticware for each step

Prepare fresh buffers for each assay

Incubation times may have been too long

Always follow the protocol and be consistent with reagent

additions and timing

Poor reproducibility between plates

Ensure all solutions are thoroughly mixed before coating the

plates

Plates may have been coated

unevenly

Seal plates after adding the coating solution to prevent

evaporation; such effects can especially be noticeable in edge

wells

Check pipettes have been calibrated and are performing as

expected

Washing may be inadequate

Increase the number and/or duration of wash steps

Confirm wells are fully emptied between washes

Plate seals may be a source of cross-

Wells may contain bubbles

contamination

Centrifuge microplates briefly prior to reading

Always use fresh plate seals between incubations





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Poor reproducibility between runs

Prepare fresh reagents (including buffers) for each assay Reagents may have degraded

Check that the standard has been prepared and stored correctly

Samples may have been handled Always store and handle samples with care and avoid repeat freezeincorrectly

thawing

Ensure all protocol steps are performed reproducibly Assay conditions may be inconsistent

Always run ELISAs under stable environmental conditions

Edge effects / plate drift

Read the plate, then rotate it by 1800 and read again; if the The plate reader may be misaligned effect remains in the same position, the reader may need to be

repaired by a qualified service engineer

Ensure all solutions are at room temperature upon addition to Solutions may be cold

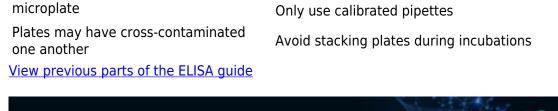
the microplate unless otherwise stated in the protocol

Prepare suitable quantities of reagents (including dead volumes) Delays may have occurred during reagent addition

for the assay to avoid running out part-way across a plate

Seal plates between reagent additions to prevent evaporation

Only use calibrated pipettes





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