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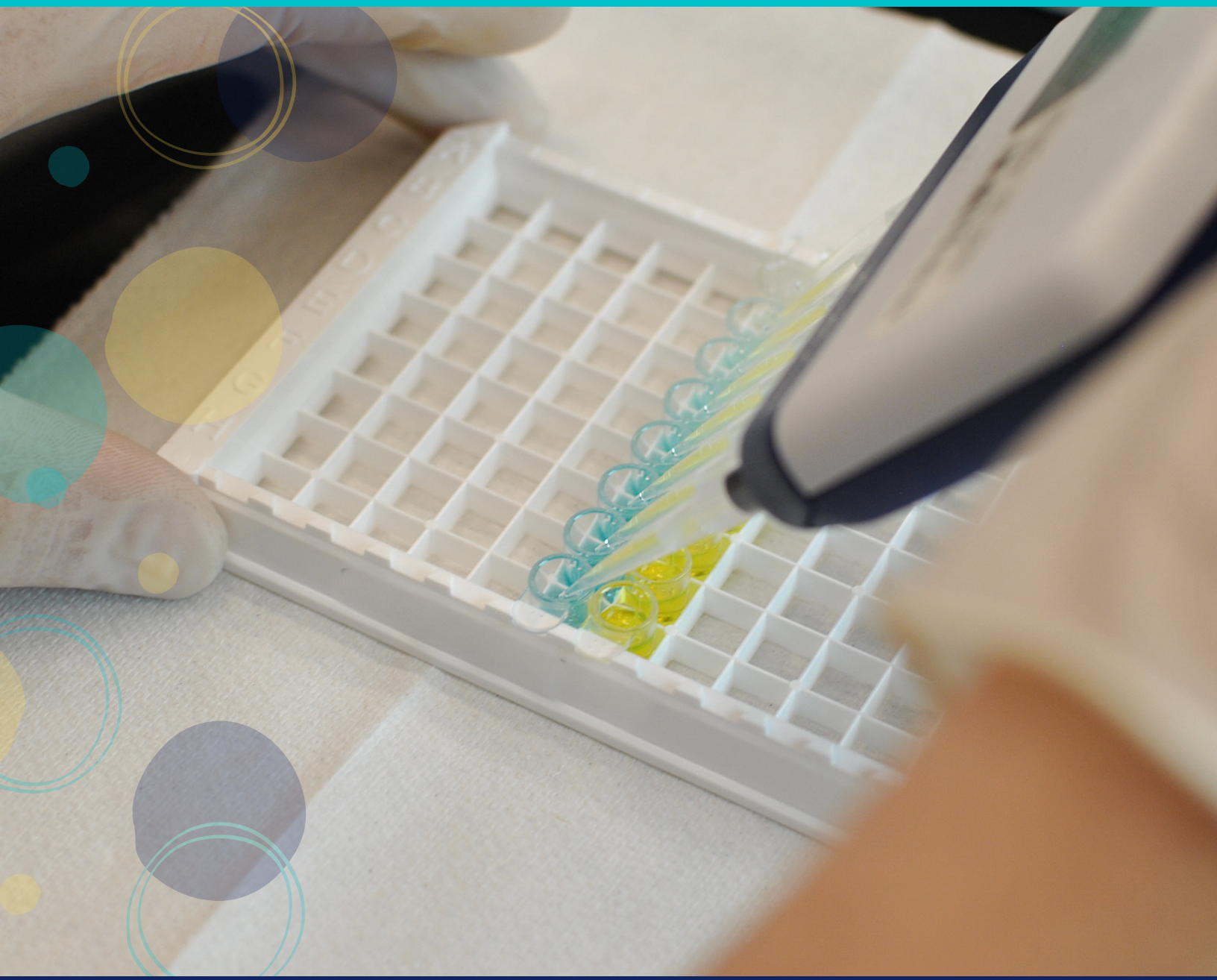
ELISA Troubleshooting Guide



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When performing an assay, there are several steps where potential issues can occur and affect your results. Here we will dive into the most common issues and how to troubleshoot them. If you encounter an issue that is not listed, feel free to contact us at service@ethosbiosciences.com or 856-224-0900. We would be happy to help!

Problem #1: High Signal

A high signal can cause the standard curve to become unusable. Here are some tips on how to prevent high signal & having to redo your assay.

Cause	Solution
Inadequate washing	Remove any residual fluid that may affect your results. Completely aspirate liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Be careful not to scratch the wells, which can also affect your results.
Incorrect dilutions	This is most likely due to a pipetting error. Check your pipetting technique.
Reaction not stopped	Make sure substrate reaction is stopped or color will begin to overdevelop.
Incubation time is too long	Make sure your incubation times are correct and adhere to the protocol within each ELISA insert.
High antibody concentration	Try different dilutions for best results.

Problem #2: Out of Range

Negative or no results after performing an ELISA can be very frustrating. Out of range results are typically due to samples & inadequate washing. However, that is not always the case.

Cause	Solution
Sample contains analyte concentrations greater than highest standard point	Samples may have to be diluted. Check the protocol.
Dilutions are incorrect	Double check your calculations and make sure your pippetting technique is correct.
Incubation time is too long	Make sure your incubation times are correct and adhere to the protocol within each ELISA insert.
Inadequate washing	Remove any residual fluid that may affect your results. Completely aspirate liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Be careful not to scratch the wells, which can also affect your results.

Problem #3: High Coefficient of Variation

Reliable assay results are assessed by standardized measures such as coefficient of variability. Data with high variation can skew your results and cause inconsistencies in your data. The most common cause of high CVs is often pipetting, but it's not always the cause.

Cause	Solution
Bubbles in wells	Double check to make sure there is no presence of bubbles prior to reading your plate. Proper pipetting can save you from having to rerun your experiment all over again.
Pipetting error	Make sure that all pipette tips are tight & calibrated.
Inadequate washing	Remove any residual fluid that may affect your results. Completely aspirate liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Be careful not to scratch the wells, which can also affect your results.
Samples may have high particulate matter	Remove matter by centrifugation.
Sample preparation	Make sure you did not forget to diltue any samples. One well of a duplicate may receive more analyte than the other if not samples are poorly mixed.

Problem #4: High Background

Did your color over-develop across your ELISA plate? The problem with high background is that it reduces the sensitivity of your assay & could possibly even ruin it completely. You do not want to use data from a plate suffering from high background. Here's how to prevent it!

Cause	Solution
Contamination of background wells	Make sure that you are avoiding cross contamination by using the sealer appropriately. Ensure that pipette tips do not touch the reagents on the plate.
Incubation time is too long	Strictly adhere to the incubation times indicated in the ELISA protocol.
Insufficient wash steps	Increase the number of plate washes & increase the soaking time in between washes.
Cross-reactivity	The detection antibody may be cross-reacting with the coating antibody on the plate.
High concentration of detection reagent	Make sure the detection reagent has been diluted properly.

Problem #5: No Signal

Negative or no results after performing an ELISA can be very frustrating. Below are listed a number of possible causes for zero signal development.

Cause	Solution
Reagents are not at room temperature	Allow reagents to sit 30-40 minutes prior to starting your assay.
Omission of reagents	Double check that you are adding the reagents in the correct order as instructed & double check your dilutions.
Incubation time too short	Check the ELISA protocol for the required incubation times.
Not enough detection reagent	Increase the concentration according to the manufacturer's guidelines.
Dried wells	Do not allow the wells to dry out during the assay.
Damaged wells	Pipette and autowasher tips can scratch the walls of the sample wells which can affect the results of your assay. Be sure to check wells are not scratched or damaged.

Problem #6: Poor Standard Curve

The standard curve is arguably the most important part of your ELISA. Low OD's (flattening of the bottom of the curve) and high OD's (flattening at the upper portion of the curve) can be frustrating. Here's some steps you can take to avoid a poor standard curve.

Cause	Solution
Incorrect standard solution	Double check your standard stock concentration and your dilutions.
Curve doesn't fit scale	Make sure you are using the manufacturers suggested curve fitting model. For example, we suggest a 4 PL fit model, however there are other alternatives for the standard curve.
Pipetting error	Check your calculations, make new standard curve. Double check that your pipettes are calibrated. You may have to repeat your assay if you have poor CVs in your standards duplicate.

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