IBL-AMERICA ELISA TROUBLESHOOTING GUIDE

PROBLEM	POSSIBLE CAUSE	SOLUTION/REMEDY
Weak or No Signal	Reagents added/prepared incorrectly	Check protocol, ensure that the reagents were added in the proper order and prepared to correct dilution.
	Reagents not equilibrated to room temperature	Per specific product protocol ensure appropriate reagents equilibrate to room temperature prior to starting the assay.
	Incorrect storage of reagents/Expired Reagents	Double check the expiration dates and storage conditions in product protocol and/or component labels.
	Very low incubation temperature or agitation.	Control your room (lab) temperature to bring it within the recommended range (Refer to the Package Insert). Set your plate shaker to 600rpm.
	Wells scratched with pipette or washing tips.	Use caution when dispensing and aspirating into and out of wells. Automated plate washers may need to be calibrated so tips don't touch the bottom of the wells.
	Plate washing to forceful	Check and ensure correct pressure in automatic plate washer. Pipette wash buffer gently if manually washing the plate.
	Plate read at incorrect wavelength	Double check product protocol to ensure plate reader is set to the correct wavelength.
	Incompatible sample type	Detection may be reduced or absent in untested sample types. Include a sample that the assay is known to detect as a positive control.
High Background Across the Plate. Standard Curve is Saturated. Too Much Signal	Insufficient washing	Wash wells as per the product protocol. As a general rule, increasing the duration of the soak step may help (Add 30 seconds to the soak time). In addition, at the end of each washing step, invert the plate on absorbent tissue and tap forcefully if necessary to remove any residual fluid.
	Contaminated wash buffer	Prepare a fresh solution of wash buffer.
	Wrong conjugate dilution used.	Dilute the conjugate at the recommended dilution.
	The assay was incubated for too long in one or all steps.	Strictly follow the assay incubation time in all steps according to the procedure.
	Substrate incubation not protected from light	Substrate incubations are commonly performed in the dark. Review the product protocol and follow recommendation of the manufacturer.
	The substrate is contaminated (not fresh).	Check the color of the substrate - it should be colorless.
	High incubation temperature.	Control your room (lab) temperature to bring it within the recommended range (Refer to the Package Insert).
	Dirty plate/well bottom	Clean the bottom of the plate.

REV1 TV052617

IBL-AMERICA ELISA TROUBLESHOOTING GUIDE

PROBLEM	POSSIBLE CAUSE	SOLUTION/REMEDY
Positive Signal (high background) in Negative Controls or Standard Wells.	Contaminated Pipette tips.	Use clean pipette tips and change tips for every sample and/or standard.
	Cross contamination has occurred between wells.	Carefully wash wells. The wash step is very critical.
	Incorrect standard dilutions prepared	Review protocol and double check calculations to ensure proper dilution of standards/controls
Poor Standard Curve in ELISA	Incorrect standard curve dilutions prepared	Confirm dilutions are made correctly and avoid cross contamination of standards by utilizing new pipette tips with each standard preparation.
	Standard improperly reconstituted	Briefly spin vial prior to opening. Inspect vial for undissolved material after reconstitution.
	Possible degradation of standard material	Ensure storage and handling of the standard was properly followed as recommended in the product protocol
	Curve doesn't fit data	Try plotting standard data using a different scale. e.g. log-log, 4 Parameter logistic, 5 parameter logistic curve fits.
High CV across the plate (high variation in samples / standards).	Pipetting problem.	When using:
		Single Channel Pipette: Check tips for bubbles between replicates.
		Multichannel Pipette: Calibrate the pipette. Check tips for bubbles before dispensing.
	Bubbles in wells	Ensure no bubbles are present prior to reading the plate.
	Edge Effects	Ensure the plate and all reagents are at room temperature.
	Insufficient washing	Wash wells as per the product protocol. As a general rule, increasing the duration of the soak step may help (Add 30 seconds to the soak time). In addition, at the end of each washing step, invert the plate on absorbent tissue and tap forcefully if necessary to remove any residual fluid.
	Non-Uniform Washing.	All the wells should be uniformly washed. Check your washing system and/or method.
	Inconsistent sample storage	Ensure sample storage conditions are maintained per guidelines outlined in the assay protocol.
	Non-homogeneous reagents, samples, or standards	Ensure all reagents are mixed thoroughly. Vortex samples/standards to ensure homogeneity before pipetting.
Sample Readings are out of the assay dynamic range.	Sample contains undetectable analysis level, i.e. below assay limit of detection (LOD).	If samples fall below the LOD, contact us: info@ibl-america.com
	Sample contains high analyte level: i.e. above assay highest standard point.	Samples should be diluted and re-assayed.

IBL-AMERICA ELISA TROUBLESHOOTING GUIDE

Pipetting Technique Tips for ELISA

1. Use only pipettes within the range suggested by the manufacturer.
2. Make sure disposable pipette tip is fit tightly onto the pipette
3. Check that there are no air bubbles while pipetting reagents/samples/standards/controls.
4. Reduce splashing of liquid by pipetting into the sides of the microplate wells.
5. Use a new pipette tip between pipetting of standards, controls, and samples

Online Software for Curve Analysis and Calculation of Results

*These programs are not affiliated with IBL-America and should be used at the discretion of the end user.

1. https://www.myassays.com/
2. http://www.elisaanalysis.com/app

If you have any additional questions please contact us directly.

8201 Central Ave NE, Suite P
Minneapolis, MN 55432, USA
info@ibl-america.com
(888) 523-1246
https://www.ibl-america.com/

REV1 TV052617