ELISA Kit Trouble Shooting Guide

International Diagnostic Training Session for DRG Distributors:

PROBLEM	*SOURCE*	SOLUTION
1) **WEAK / NO COLOR DEVELOPMENT**	• Substrate was not added, or was added at the wrong time.	➢ **Follow kit insert protocol and add Substrate.**
	• Antibody or Conjugate was not added, or was added at the wrong time.	➢ **Follow kit insert protocol and re-run assay.**
	• Components were used which do not belong to the specific kit being used	➢ **Use only lot-specific components**
	• Plate was not incubated, or Stop Solution was added prior to the final incubation.	➢ **Re-run assay**
	• Substrate has been contaminated.	➢ **Use new substrate**
	• Incorrect storage of components, e.g., not stored at +2-8°C.	➢ **Store all components exactly as directed in protocol and on labels.**
	• Omission of any incubation steps	➢ **Follow incubation outlined in the protocol.**
	• Standards diluted in serum, culture medium, or other solution.	➢ **Follow kit insert protocol.**
	• Did not add the proper volumes of reagents.	➢ **Re-run assay with recommended volumes.**
	• Did not incubate the plate for the proper amount of time and /or temperature.	➢ **Use recommended incubation time and temperatures.**
	• Reagents were not brought to room temperature prior to use.	➢ **Re-run assay with room temperature reagents.**

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	• There is a contaminant in one or all of the reagents.	➢ **Use new reagents**
	• The plate was not read immediately after Stop solution was added.	➢ **Read plate within 10 minutes of Stop Solution addition or re-run assay.**
	• Incorrect TMB /Stop solution used	➢ **Use only the TMB and stop solution contained in the kit.**
	• Excessively cool laboratory temperature	➢ **Adjust laboratory temperature (Room temperature)**
	• Reagents have expired	➢ **Check expiration dates upon receipt of kit and use kit prior to expiration.**
	• Plate read at incorrect wavelength.	➢ **Follow the kits insert protocol.**
	• Attempt to measure analyte in a matrix for which the assay has not been optimized.	➢ **Please contact Technical service for advice when using non-validated sample types.**
2) POOR STANDARD CURVE	• Improper dilution of standards	➢ **Re-run assay**
	• Reagents were not brought to room temperature prior to use.	➢ **Re-run assay with room temperature reagents.**
	• Unequal volumes of standard used	➢ **Check pipet calibration and re-run assay.** ➢ **Pre-rinse pipet tip in reagent before transfer.**
	• Wells were not washed properly	➢ **Make sure the wash apparatus is functioning properly or follow kit insert protocol.**
	• Wells were not aspirated properly	➢ **Make sure wells are dry after aspiration.**

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	• Incorrect incubation times and/or temperatures	➢ **Use recommended incubation times and temperatures.**
	• Pipetting error	➢ **Check pipet calibration and re-run assay.** ➢ **Pre-rinse pipet tip in reagent before transfer.**
	• Calculations were not done correctly	➢ **Follow kit insert directions on how to perform the appropriate calculations.**
	• Improper preparation of standard stock solution.	➢ **Dilute lyophilized standard as directed by the vial label or protocol.**
	• To flat or too steep calibration curve may be obtained when incubation time is too long respective when incubation temperature is too high.	➢ **Follow manual Instruction**
	• As a rule of thumb the following should be considered: • 6 minutes longer or shorter incubation times give an approx. 10% higher or lower signal.	➢ **Follow manual Instruction**
	• Inadequate washing and draining of wells	➢ **Wash according to the protocol. At the end of each wash step, invert plate on absorbent tissue on countertop, allow not completely drain, and tap forcefully if necessary to remove residual.**
	• Errors in pipetting the standard or subsequent steps	➢ **Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device.**

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	• Reagents (lyophilized standard, standard diluent, buffer, etc.) From different kits, either different lot number, were substituted.	➢ **Never substitute any components from another kit.**
3) POOR PRECISION	• Bad precisions result from different incubation temperatures, e.g. The way the sun`s rays fall (warming up) or one-sided warming-up.	➢ **During hot summers when temperatures are usually high (over 27°C) the incubation times should be respectively shortened according to the manual instructions.**
	• 1°C difference in temperature of the incubation temperature shows for example approx. 80-150 mu higher or lower extinctions.	➢ **During hot summers when temperatures are usually high (over 27°C) the incubation times should be shortened according to the manual instructions.**
	• Wells were not washed properly.	➢ **Make sure the wash apparatus is functioning properly or follow kit insert protocol.**
	• Wells were not aspirated properly.	➢ **Make sure wells are dry after aspiration.**
	• Pipetting error	➢ **Check pipet calibration and re-run assay.** ➢ **Pre-rinse pipet tip in reagent before transfer.**
	• Errors in pipetting the standard, samples or subsequent steps	➢ **Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device.**
	• Wells have been scratched with pipette tip or washing needles.	➢ **Use caution when dispensing and aspirating into and out of microwells.**
	• Incorrect volumes of	➢ **Follow protocol for reagent dispensing**

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	materials dispensed into microwells.	**volumes. Check calibration of pipettes.**
	• Standard was diluted with the serum, culture medium or other buffer.	➢ **Follow kit insert protocol.**
	• Particulates or precipitates found in the samples.	➢ **Remove any particulates/precipitates by centrifugation prior to dispensing into the assay.**
	• Dirty microwells-visible debris within or on bottom of microwells.	➢ **Inspect microwells and invert plate to remove debris but only outside. Wipe bottom of plate with an absorbent tissue after each wash step. Never insert tissue into microwells.**
4) EDGE EFFECTS	• Temperature is uneven in the work area.	➢ **Work in an area free of drafts and temperature fluctuations.**
	• Plates have been stacked on top of one another during incubation.	➢ **Do not stack plates.**
	• Plate not properly sealed for non-room temperature or 37°C incubation.	➢ **Make sure that edge and corner wells are sufficiently sealed with plate sealer.**
5) UNEXPECTED RESULTS	• There are interfering factors in your samples.	➢ **Determine the source of interference and omit it.**
	• Wave length of the photometer is not longer recorded properly due to filter fading.	➢ **Unit should be calibrated regularly. If necessary, filters should be changed.**
	➢ Wrong kit has been taken from the program.	➢ **Check if the kits has been clicked on the programme and, if need be, measure again.**

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6) HIGH BACKGROUND	• Plate was not washed properly.	➢ **Make sure the wash apparatus is functioning properly.** ➢ **Make sure all wash buffers is removed before adding substrate.**
	• Substrate has been contaminated	➢ **Use new substrate**
	• Plate was incubated too long, or at the wrong temperature.	➢ **Use recommended incubation times and temperature.**
	• Pipet tips have been handled without glove	➢ **Use only clean gloves to handle pipet tips, or use pre-packed pipet tips.**
	• Insufficient washing and/or draining of wells after washing.	➢ **Wash according to the protocol. At the end of each washing step, invert plate on absorbent tissue on countertop and allow to completely drain and tap forcefully if necessary to remove residual.**
	• Contamination of substrate solution with metal ions or oxidizing reagents.	➢ **Use distilled/deionized water for dilution of wash buffer and use plastic equipment.**
	• TMB exposed to light prior to use, resulting in a blue color.	➢ **Keep chromogen in vial until ready to dispense into plate and then pour into a reservoir to prevent contamination of the vial with equipment.**
	• Incorrect incubation times and/or temperatures	➢ **Follow protocol**
	• Evaporation of wells during incubations.	➢ **Securely seal plate with plate cover during all incubations.**

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7) **DRIFT**	• Set-up of the assay has been interrupted.	➢ **Minimize interruptions and have standards and samples prepared before starting the assay.**
	• Reagents were not brought to room temperature prior to use.	➢ **Re-run the assay with room temperature reagents.**
8) ELEVATED SAMPLE / STANDARD ODs	• Standards diluted in serum, culture supernatant or other.	➢ **Follow kit insert protocol**
	• Incubation time extended	➢ **Follow incubation times outlined in protocol.**
	• Incubation carried out at 37°C when RT is recommended.	➢ **Perform incubations at Room temperature when instructed in the protocol.**
	• Substrate exposed to light prior to use	➢ **Exposure of the chromogen to light causes the solution to change from clear to blue. Keep the chromogen in vial until ready to dispense into plate and then pour into a reservoir to prevent contamination of the vial with equipment.**