ELISA Troubleshooting Guide:

Problem	Probable Cause	Solution
Signal is high, standard curves have saturated O.D.'s	Standard reconstituted with less volume than required	Reconstitute lyophilized standard with correct volume of solution recommended in the protocol.
	Plate incubation was too long	Decrease incubation time.
	Detection antibody incubation time is too long	Decrease detection antibody incubation time.
	Avidin-HRP incubation time is too long.	Decrease Avidin-HRP incubation time.
	Substrate solution incubation time is too long	Decrease substrate solution incubation time.
Sample readings are out of range	Samples contain no or below detectable levels of analyte	If samples are below detectable levels, it may be possible to use higher sample volume. Check with technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point.	Samples may require dilution and reanalysis.
High variation in samples and/or standards	Multichannel pipette errors	Calibrate the pipettes.
	Plate washing was not adequate or uniform	Make sure pipette tips are tightly secured. Confirm all reagents are removed completely in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before pipetting.
	Samples may have high particulate matter	Remove the particulate matter by centrifugation.
	Insufficient plate agitation	The plate should be agitated during all incubation steps using an ELISA plate shaker at a speed where solutions in wells are within constant motion without splashing.
	Cross-well contamination	When reusing plate sealers check that no reagent has touched the sealer. Care should be taken when using the same pipette tips used for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

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Background is high	Background wells were contaminated	Avoid cross-well contamination by using the sealer appropriately. Use multichannel pipettes without touching the reagents on the plate.
	Matrix used has endogenous analyte or interference	Check the matrix ingredients for cross reacting components (e.g. interleukin modified tissue culture medium).
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue.
	Avidin-HRP was not added.	Add Avidin-HRP according to protocol and continue.
	Substrate solution was not added.	Add substrate solution and continue.
	Wash buffer contains sodium azide	Avoid sodium azide in the Wash Buffer.
Low or poor signal for the standard curve	Standard was incompletely reconstituted or was inappropriately stored	Reconstitute standard according to protocol. Store reconstituted standard in appropriate vials. Store reconstituted standard at -70°C.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and correct reagent volume.
	Incubations done at inappropriate temperature, timing or agitation	Assay conditions need to be checked.