High Backgrounds (NSB)

Elevated background or non-specific binding (NSB) in an ELISA as evidenced by high absorbances in the zero standard can be due to a number of problems as discussed below. Keep in mind that different assays may have widely different expected absorbances for the zero standard, due to inherent design features of that assay. For example, HCP assays tend to have much higher background NSB than assays for single analyte. This is because HCP assays typically must use on the order of 20 to 50 times the amount of enzyme:antibody conjugate compared to single analyte assay such as our kits for Protein A or BSA. To determine the expected absorbance for the zero standard in your particular kit lot, please refer to the Certificate of Analysis that comes with each shipment. This COA will show the absorbance for the zero standard obtained by our QC laboratory.

1. Washing Procedure – Incomplete washing of the wells can result in carryover of unbound reagent and thus high and variable background. Review the kit package insert for proper washing technique and review "Washing of Microtiter Wells" to get a more detailed explanation of the recommended washing technique. Use only the diluted wash concentrate solution provided with the kit as other formulations, particularly those with detergent, may increase NSB. Do not wash plates more than 4 times or allow the wash solution to soak in the wells for any period of time as this will reduce specific binding for the assay. Or you can review the "Washing Technique for Microtiter Plate ELISA Video" to view the washing technique we recommend.

2. Contamination of kit reagents by concentrated sources of the analyte in your laboratory can also result in high NSB. Please refer to the article "Avoiding Contamination of Kit Reagents" for a more detailed discussion on how to prevent contamination. Our ELISAs are very sensitive methods, capable of measuring analytes in the pg/mL to ng/mL range. Many laboratories will have sources of the analyte in question at very high concentrations near to where they perform the ELISA. For example, culture media or samples from very upstream in the purification process may have HCPs or growth media additives like BSA in the mg/mL range. Such upstream samples can have more than a million-fold greater concentration than the sensitivity of the assay for that analyte. In such cases it is easy to contaminate some of the kit reagents such as random microtiter plate wells, a standards' vial, or conjugate bottle.

3. Contamination of the substrate used in the kit can also result in high NSB. This is most often seen with alkaline phosphatase-based ELISA using PNPP substrate. It is rarely a problem in HRP based assays with TMB as the substrate. Review the package insert for techniques to minimize contamination of PNPP substrate. If your substrate has become contaminated, you should order replacement substrate.