



Sample Matrix Interference in ELISA, How to Overcome It.

It is not uncommon to encounter sample matrix interference when testing for bioprocess contaminants in your various sample types. Such interference can manifest itself as either falsely depressed or falsely elevated levels of analyte. While the product protein itself can be responsible for this interference, other components of the sample matrix such as buffer salts, pH, or other additives like detergent or solvents are often problematic. Experiments to test for sample matrix interference can be found in the sections on [Spike & Recovery Studies](#) and [Dilutional Linearity](#).

There are a number of ways to solve sample matrix interference some of which are described below. Should you still encounter problems after trying the following options, we suggest you to contact our Technical Service Department for assistance.

1. One of the easiest ways to overcome sample matrix interference is by diluting the sample into a more assay compatible buffer. The ideal diluent is the same material used to prepare the kit standards. As the sample is diluted its matrix begins to approach that of the kit standards and as such tends to improve the specificity and accuracy of the assay. Cygnus sells assay specific diluents in volumes of 100mL, 500mL and 1L bottles. Sample dilution is only an option if your samples either: a) have levels of analyte that remain within the analytical range of the assay after dilution or b) if the allowable limits of the analyte contaminating your product do not fall below the LOQ of the assay after the necessary dilution. Provided a Minimum Required Dilution (MRD) can be experimentally established for your sample types, dilution is the preferred method for solving sample matrix problems.
2. When dilution is not an option because it does not solve the problem or because of assay sensitivity issues, consider sample processing to remove the interfering components. A very effective technique is to perform a buffer exchange of your samples into a more assay compatible matrix. A very easy way to do this is to use small pre-calibrated Sephadex G-25 columns sold by Amersham Biotech called NAP 10 Columns. The procedure is to first equilibrate the column in assay diluent (the same material used to prepare the kit standards). Apply 1mL of your sample to the column and let it flow into the column; at which point the flow will automatically stop due to surface tension. Next apply 1.5mL of diluent buffer and collect the 1.5 mL that elutes from the column. This 1.5mL will contain greater than 98% of the starting proteins in your sample including product and contaminants like HCPs but without greater than 98% of the low molecular weight components such as buffer salts etc. Your sample has only been diluted by a factor of 1.5 but it is now in the ideal assay matrix and thus should allow for good analytical accuracy.
3. Consider simple neutralization of your samples to overcome pH problems. In general, ELISA may not work well if the sample pH is outside of the neutral range, <6.0 or >8.5. Addition of a buffering concentrate can neutralize your sample to the ideal pH of 7.0-7.5 without much dilution of the sample.

4. Modification of the ELISA protocol can also minimize sample matrix interference. Less sample size, longer incubation times, or use of a simultaneous incubation protocol in which the sample is incubated in the coated capture well simultaneously with the enzyme conjugated antibody can also be effective. Please refer to [Modification of ELISA Protocol](#) for more information.