

Dilution of Samples, Issues to Consider

Some samples, particularly those from upstream in your purification process will have impurity analyte concentrations above the analytical range of our very sensitive ELISA kits. Such samples may require very large dilutions in order to overcome “Hook Effect” and to achieve acceptable “sample dilution linearity”. Please refer to “Poor Dilution Linearity” for a more detailed discussion on sample dilution issues. In addition to the “Hook Effect,” the matrix of some samples may interfere non-specifically with the assay and result in under recovery of the true analyte levels. Simple dilution of those samples is often adequate to buffer out such interference provided the dilution does not reduce analyte concentrations below the limit of quantitation of the assay. In cases where dilution of your samples is not an option, contact our Technical Service Department for advice on how best to overcome sample matrix interference.

Cygnus offers assay specific diluents for each of its kits. The catalog numbers for these diluents can be found in the kit product insert or by contacting Customer Service. We strongly recommend use of those diluents because they are the same formulation as the matrix used for the kit standards. Thus, as you dilute your samples in our diluent, your sample matrix begins to approach that of the standards and in this way greatly minimizes any dilutional artifacts that could occur if you were to use another diluent.

If you elect to use another diluent you must validate that it provides accurate results. We recommend two critical experiments for validation:

- First, you must assay the diluent alone to determine that it does not yield absorbance values significantly above or below the absorbance for the kit zero standard. OD values above the zero standard indicate that the diluent may have low levels of the analyte or that the diluent causes an increase non-specific binding, relative to the kit standards matrix. OD values less than the kit standard may indicate that the proposed diluent is lowering non-specific binding and may also be inhibiting specific binding. In any event use of a diluent that does not match the standards risks significant errors when the sample concentration gets multiplied by the dilution factor.
- Second, you must perform a spike & recovery experiment into your proposed diluent at several levels across the analytical range of the assay. For a diluent to be deemed acceptable we suggest a recovery specification of 95% to 105%. In general, the formulation of diluents should be a neutral pH with some carrier protein added to block non-specific adsorptive losses of the analyte. **Use of just PBS or TBS without a carrier protein can be problematic because the analyte diluted in the range of the assay (ng/mL) can very significantly adsorb to the dilution tube resulting low recovery!** Your diluents should not have sodium azide as a preservative or significant detergent concentrations as these will reduce assay accuracy.