

## Dilutional Linearity

All sample types with apparent levels of analyte greater than the LOQ of the assay should initially be evaluated for dilutional linearity as part of assay validation. This experiment involves performing a number of serial dilutions using an approved assay diluent. These dilutions are then assayed and a dilution corrected contaminant concentration is determined at each dilution. This dilutional linearity study establishes freedom of sample matrix interference and also demonstrates the important condition of antibody excess for the array of contaminants in your samples. Please refer to the section on "[Hook Effect](#)". If you will be routinely testing in-process samples in addition to final product, you should validate dilutional linearity of each sample type. This analysis is critical for HCP assays because very high concentrations of certain HCPs may approach saturation of the antibody against that particular HCP. When this happens, there is a risk of significant under-quantitation for that HCP. By performing dilutional analysis, one can verify if the antibody is in excess and that the sample matrix itself does not interfere. If the antibody is in a limiting concentration or the sample matrix causes a negative interference you may observe that the apparent HCP concentration for a sample *increases* with *increasing* dilution.

In most cases a dilution will be reached where the dilution corrected value remains essentially constant. We term this the Minimum Required Dilution or MRD. The table below shows example data where a sample did not yield good dilutional linearity at high concentration but with further dilution an MRD was determined at which acceptable dilutional linearity was obtained. In this example we conclude that the MRD for this in-process sample is 1:8 and that the concentration of HCP to be reported is 361ng/mL. Once an MRD is established for a particular sample type, your SOP should reflect that these samples need to be diluted before assaying. We suggest defining acceptable dilutional linearity as dilution corrected analyte concentrations that vary no more than 80% to 120% between doubling dilutions. Due to the statistical limitations in the low end of the assay range, you should avoid considering dilutional data where the assay value before dilution correction, falls below two times the LOQ of the assay. Acceptable diluents may vary from assay to assay and you are encouraged to verify with *Cygnus Technologies* that your sample diluent is acceptable.

In general, the best diluent is the same one used to prepare the kit standards. Assay specific diluents can be purchased from *Cygnus* in 100ml, 500ml or 1000mL bottles. Contact *Cygnus* for information on acceptable diluents. Should you determine that there is significant product or matrix interference and simple dilution is not an option, it may be necessary to further process the sample by methods such as buffer exchange to render it into a more assay compatible buffer. In other cases, modification of the assay protocol can affect improved accuracy in some sample types. Users of our kits are encouraged to contact the Technical Services Department for advice on how best to solve sample accuracy issues.

Example Dilutional Linearity Data

Sample Dilution	Dilution Corrected Value (ng/mL)	% change in concentration from previous dilution
Neat (undiluted)	146	NA
1:2	233	160%
1:4	312	134%
1:8	361	116%
1:16	356	99%
1:32	370	104%
1:64	ND < LOQ of assay	NA