

Quality Control of ELISA

Analyte Controls –

The routine run-to-run quality control of ELISA is best accomplished by assaying control samples across the important analytical range of the assay. We recommend 3 controls, a low level control in the range of 1 to 2 times the assay LOQ, a medium level control, and a high level control. These controls should be made using your source of analyte (e.g. HCPs from your cell line, your growth media, or samples from your purification process). Furthermore the controls should ideally be in the same matrix as your critical samples. By using your source of analyte in your sample matrices you will be best able to identify any problems within the run or between runs and kit lots. *Cygnus* manufactures its kits for lot-to-lot consistency and tries to avoid changes in any components or procedures that could impact accuracy in the customer laboratory. However, as generic kits, we can only quality control them by a limited range of parameters that may or may not be sensitive to your product specific issues. **Use of laboratory specific controls is the only way to assure total quality control of the assay for your needs.** Controls should be made in bulk, aliquoted for single use, and frozen at -80°C until stability studies indicate some other storage conditions are adequate. Once you have statistically established a range for these samples they will become the most sensitive and specific tool to assure quality control of the assay. **Do not rely on curve fit parameters as quality control specifications in the absence of true analyte controls. Curve fit parameters such as R^2 , slope, y-intercept, and upper and lower asymptotes are not sensitive or specific enough to reliably detect assay problems.** Use of those parameters in the absence of true analyte controls will frequently fail a perfectly good run and worse cause you to pass a run that would have been flagged had analyte controls been used. Contact our Technical Services Department for advice on how to make and establish controls specific to your needs.

Numbers of Replicates -

When precision is very good (average replicate %CVs on ODs are less than 5%) we feel duplicate analysis is adequate and the most cost effective approach. In the case of duplicate analysis we do not allow for editing of any apparent outliers since there is no statistical basis for establishing which of the duplicates is inappropriate. Thus in duplicate analysis we suggest repeating any sample that yields a %CV greater than 20%. Alternatively, performance of the assay in triplicate or even quadruplicate may allow for editing of data points such that it is unnecessary to perform a repeat assay. Criteria for deleting certain data points are somewhat subjective but should take into consideration the impact of your error limits on product safety or allowable levels of contaminant.