

Spike & Recovery Studies

In some cases your product itself or certain components in the product formulation buffer may interfere (either positive or negative interference) in the ability of the assay to detect HCPs or other contaminants. Similarly, samples from upstream in the purification process may also contain material in their matrices that can interfere in ELISA methods. Factors such as extremes in pH, detergents, organic solvents, high protein concentration, and high buffer salt concentrations are known interference components. For these reasons it is necessary to validate by universally recognized experimental procedures (i.e. ICH & FDA guidelines) that the assay will yield accurate results. The two critical experiments in assessing assay accuracy and specificity are 1) Spike & Recovery and 2) Sample [Dilutional Linearity](#). Should the end user of this kit determine that there is significant product or matrix interference, it may be necessary to further process the sample by methods such as dilution or buffer exchange to render it into a more assay compatible buffer. The same diluent used to prepare the kit standards is ideally the preferred material for dilution or buffer exchange of your samples. In other cases, modification of the assay protocol can improve accuracy in some sample types.

For each sample type to be tested, be it final product or in-process samples, you should demonstrate that the assay can recover added HCP or other contaminants spiked into that sample matrix. This can be performed by spiking the highest standard provided with the kit into your sample types and then testing in the assay. Using the *E. coli* HCP kit, Cat # F010 as an example, we suggest spiking 1 part of the 250ng/mL standard into 4 parts of your sample (e.g. spike 100 μ L of 250ng/mL standard into 400 μ L of sample). The spiked concentration into the sample in this case is 50 ng/mL. A control dilution of 1 part of assay diluent (zero standard) to 4 parts of sample is also performed to determine the contribution of endogenous HCP in the sample prior to spiking. Both the spiked and diluted, unspiked sample are assayed. Percent added recovery is determined by subtracting the endogenous contribution of HCP from the total HCP measured in the spiked sample. We suggest acceptable recovery should be within 80% to 120% of the spiked HCP. The table below shows example data. If you desire spike and recovery at more than one concentration we recommend that the lowest spike levels should be at least 2 times the Limit of Quantitation (LOQ) of the assay and that the contribution of the endogenous HCP in the sample prior to spiking not exceed two times the spike level to be tested. These two conditions will insure better statistical accuracy.

Example Spike and Recovery Data

| Sample | Spike Conc. (ng/mL) | Total HCP measured (ng/mL) | % Spike Recovery |
|--|---------------------|----------------------------|------------------|
| 4 parts final product + 1 part zero standard | 0 | 22 | NA |
| 4 parts final product + 1 part 250ng/mL std. | 50 | 70 | 96% [(70-22)/50] |