

## Poor Spike and Recovery

Spike and recovery of known amounts of analyte into your various sample types is a critical experiment to validate the accuracy of a given method. In some cases the product protein itself or certain components in the product formulation buffer may interfere in the ability of the assay to detect HCPs or other contaminants. Factors such as extremes in pH, detergents, organic solvents, high protein concentration, and high buffer salt concentrations are known to interfere. This interference is normally negative in nature and manifests itself as under recovery of the spiked analyte. It is necessary to validate by universally recognized experimental procedures (i.e. ICH & FDA guidelines) that the assay will yield accurate results. Should the end user of this kit determine 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 affect improved accuracy in some sample types. You are encouraged to contact our Technical Services Department for advice on how best to solve poor spike and recovery problems in our kits.

For each sample type to be tested, be it final product or in-process samples, you should demonstrate that the assay can recover added analyte spiked into that sample matrix. This can be simply performed by spiking some of 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 $\mu$ L of 250ng/mL standard into 400 $\mu$ 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. Table 1 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.

**Table 1**  
**Example Spike and Recovery Data**

<b>Sample</b>	<b>Spike Conc.</b>	<b>Total HCP measured</b>	<b>% Spike</b>
---------------	--------------------	---------------------------	----------------

	(ng/mL)	(ng/mL)	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]