



**SP 2/0 HCP ELISA kit
Catalog # F180
Validation Summary
Report dated Jan. 24, 2001**

The data summarized below was generated by *Cygnus Technologies* to establish the performance parameters and validity of this kit to measure SP 2/0 Host Cell Proteins (HCPs). This data is intended to supplement and not replace user generated validation data. The data is representative of what a laboratory can expect to achieve when following the kit insert recommended protocols. Significant differences in these performance parameters may be indicative of problems with reagents, laboratory equipment, or technique and should be investigated before reporting results.

It is recommended that a user validation study include at least the following experiments to validate this kit for use with their product: (1) Each user should perform intra and inter assay precision experiments to establish their procedural proficiency. (2) Each user should perform recovery experiments using their test sample matrices. Such a study can be performed by adding known amounts of the 200ng/mL standard provided with this kit to the final product or any intermediate samples, which are to be tested. Ideally these test sample matrices should be devoid of any SP 2/0 proteins or have very low levels (<4ng/mL) determined prior to adding the 200ng/mL standard. Such an experiment will establish the degree of sample matrix interference in the recovery of HCPs. (3) Laboratories should also perform dilutional recovery experiments on their actual samples. This experiment assumes that at least some of the test samples from the purification process will have significant levels of HCPs. Such samples are to be serially diluted by some appropriate diluent previously shown to give acceptable recovery. When diluted, samples should give essentially the same value at each dilution when multiplied by the appropriate dilution factor. This experiment establishes the condition of antibody excess for accurate quantitation and determines that typical process samples do not have HCPs in the “Hook Region” of the concentration response curve.

Materials: Kit Lots # 14120 & 11011 were used for all studies.

Methods: Assay protocol as specified in kit directions insert.

Data References: Raw data for these experiments are recorded in Notebook #SP2-01.

Precision: Precision is defined as the percent coefficient of variation (%CV). This is calculated by dividing the mean by the standard deviation for a number of replicate determinations of three different control samples in the low, medium and high concentration range of the assay. The design goal specifications are given in the last column of each experiment. While actual precision may vary from laboratory to laboratory, and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Intra-assay				Inter-assay			
# of tests	Mean ng/mL	%CV	Design Goal Specification	# of assays	Mean ng/mL	%CV	Design Goal Specification
10	8.8	6.8	<12%	5	8.3	8.1	<15%
10	25.2	1.6	<10%	5	24.6	4.3	<10%
10	67.4	1.8	<10%	5	72.2	5.5	<10%

Recovery/Matrix Interference: The same SP 2/0 HCP preparation used for the standards was spiked into various “sample buffers” to demonstrate the potential for matrix interference. HCPs were added at 2, 20, and 200ng/mL and tested in duplicate. The average % recovery is reported in the last column. Acceptable recovery is specified as plus or minus 20% of the added HCP value. These data serve as examples of certain buffers or buffer components, which may or may not give matrix interference. Matrix interference can be either positive (false increase in HCPs) or negative (false decrease in HCPs). This assay has been designed to minimize matrix interference but it is strongly recommended that users test their sample matrices for recovery in a similar experiment.

Sample Buffer Matrix	Average % Recovery (assayed/added x100)
0.05M TBS with 12.5mg/mL human IgG, pH 7.2	88
0.05M TBS with 1mg/mL human IgG, pH 7.2	98
Citrate/Phos. with 1mg/mL human IgG, pH 6.0	99
Citrate/Phos. with 1mg/mL BSA, pH 6.0	109
0.05M PBS with 12.5mg/mL human IgG, pH 7.0	91

Sensitivity: The SP 2/0 HCP concentration corresponding to a signal 2 standard deviations above the mean of the zero standard is defined as the limit of detection (LOD). This was determined from 10 replicates of the zero standard. The mean signal of the zero standard plus 2 SD yielded a LOD of ~ 1ng/mL. The limit of quantitation (LOQ) is defined as the lowest concentration for which the CV is typically <20%. This is determined by performing a precision profile for the assay at several low concentration points and then interpolating that concentration which corresponds to a 20% CV. The LOQ was 2ng/mL.

Specificity: Normal mouse and normal human IgGs were tested for cross-reactivity at 10mg/mL and at 10µg/mL, and found to be non-reactive in this assay. Other proteins have not been tested for cross reactivity. Each user should determine if other known constituents in their samples are cross reactive or interfere in any way at their typical concentrations.

Cross Reactant	% Cross Reactivity
Mouse IgG at 10mg/mL	0
Mouse IgG at 10µg/mL	0
Human IgG at 10mg/mL	0
Human IgG at 10µg/mL	0

Hook Capacity: Very high concentrations of HCPs (>200ng/mL) were evaluated for the hook effect. At concentrations exceeding 200µg/mL, the apparent concentration of SP 2/0 HCPs may read less than the 200ng/mL standard. Samples yielding signals above the 200ng/mL standard or suspected of having concentrations in excess of 200µg /mL, should be assayed diluted.