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**HEK 293 HCP ELISA kit
Catalog # F150
Validation Summary
Report dated Dec 7, 1999**

The data summarized below was generated by *Cygnus Technologies* to establish the performance parameters and validity of this kit to measure HEK 293 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 250ng/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 HEK 293 proteins or have very low levels (<4ng/mL) determined prior to adding the 250ng/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 will 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 Lot # 6129, Exp. Date 04/30/00 used for all studies.

Methods: Assay protocol as specified in kit Directions Insert revision #13-99.

Data References: Raw data for these experiments are recorded in Notebook #HEK-11.

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
20	3.3	9.6	<12%	5	3.6	14.8	<15%
20	34.5	7.1	<10%	5	42.6	7.7	<10%
20	113.6	6.8	<10%	5	125	5.4	<10%

Recovery/Matrix Interference: The same HEK 293 HCP preparation used for the standards was spiked into various “sample buffers” to demonstrate the potential for matrix interference. HCPs were added at 10, 33, and 100 ng/mL and tested in duplicate. The average % recovery is reported in the last column. The 0.1M Acetate, 1% triton, 10mg/mL BSA at pH 5.0 gave an apparent value of 4.8ng/mL when no HEK HCPs were added. This apparent activity was subsequently shown to be due to an increase in non-specific binding and not the presence of true immunological activity. In all other cases the zero for each sample buffer was within the limit of detection for the assay and thus the buffers themselves were considered to contribute 0 ng/mL of HCPs. 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. As shown below, 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 10mg/mL BSA, pH 7.2	110
0.05M PBS pH 7.2	90
Citrate/Phosphate with 10 mg/mL BSA, pH 6.0	108
Citrate/Phosphate with 1 mg/mL BSA, pH 6.0	109
0.05M TBS with 1% Tween 20, pH 7.2	81
0.1M Acetate, 10mg/mL BSA, 1% Triton, pH5.0	106
0.05 M Tris, 10mg/mL BSA, pH 8.5	116

Sensitivity: The HEK 293 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 ~270pg/mL. The limit of quantitation (LOQ) is defined as the lowest concentration for which the CV is <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 1ng/mL.

Specificity: As indicated in the table below, 4 human proteins were tested for cross-reactivity at two different concentrations. No reactivity was seen for albumin, transferrin or insulin. Very low reactivity was detected for human IgG. It could not be determined if this apparent cross reactivity is with IgG itself, or some trace impurity in the IgG, or if it represents some non-specific interaction between human IgG and the antibodies used in this kit. Other proteins have not been tested for cross reactivity. Each user should determine if other known constituents in their samples should be tested for cross reactivity.

Cross Reactant	% Cross Reactivity
Human Albumin at 5 mg/mL	0
Human Albumin at 5 µg/mL	0
Human Transferrin at 2 mg/mL	0
Human Transferrin at 2 µg/mL	0
Human Insulin at 75 µg/mL	0
Human Insulin at 750 ng/mL	0
Human IgG at 5 mg/mL	0.008
Human IgG at 5 µg/mL	0.014

Hook Capacity: Very high concentrations of HCPs (>250ng/mL) were evaluated for the hook effect. At concentrations exceeding 10,000ng/mL, the apparent concentration of HEK 293 HCPs may read less than the 250ng/mL standard. Samples yielding signals above the 250ng/mL standard, or suspected of having concentrations in excess of 10,000ng /mL should be assayed diluted.