

CHO HCP ELISA Assay

Validation Summary Catalog # F015

Summary and Explanation

The data summarized below was generated by Cygnus Technologies to establish the performance parameters and validity of this kit to measure CHO 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 CHO 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 are to be serially diluted with Sample Diluent Buffer, Catalog # I028 the approved diluent for this assay, or 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 & Methods Used

Materials	
CHO HCP ELISA Kit, Lots #K146, 15038, 12119	Cat #F015
The protocol as defined in the kit insert was used in this validation.	#5-99
Data References: Raw data for these experiments are recorded in Cygnus Notebook.	#1-CHO

Precision

Precision is defined as the percent coefficient of variation (%CV). This is calculated by dividing the standard deviation by the mean value for a number of replicate determinations of three different control samples in the low, mid 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:

# of tests	Mean ng/mL	%CV	Design Goal Specification
20	4.2	6.7	<12%
20	200	5.8	<10%

Inter-assay:

# of assays	Mean ng/mL	%CV	Design Goal Specification
5	4.5	8.4	<15%
5	19.5	5.9	<10%

Sensitivity

The CHO 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. Using the standard protocol, the mean signal of the zero standard plus 2 SD yielded a LOD of ~400pg/mL. The LOD of the rapid protocol was 1.1ng/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 450pg/mL for the standard protocol and 1.4ng/mL for the rapid protocol.

Specificity

The antibodies used in this kit demonstrated by Western Blot that they each recognize more than 40 distinct bands from CHO cells on one dimensional SDS electrophoresis under reducing conditions. The antibodies were generated against a blend of two CHO cell lines commonly used in recombinant procedures; cell line ATCC CRL-9618 and ATCC CRL-1781. Cross reactivity has not been extensively evaluated with this kit. Since the

antibody was generated to a CHO cell lysate it is possible that some of the antibodies will cross-react to conserved proteins from other animals. Apparent immunological cross reactivity should be distinguished from non-specific binding (NSB). Consult with Cygnus Technologies technical service department for advice on how to assess cross reactivity versus NSB.

Company Information

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Recovery/Matrix Interference

The same CHO HCP preparation used for the standards was spiked into various "sample buffers" to demonstrate the potential for matrix interference. HCPs were added at 2, 8, and 20ng/mL and tested in duplicate. The average % recovery is reported in the last column. In all 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 0ng/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	% Recovery (assayed/added x100)
0.05M TBS with 10mg/mL BSA, pH 7.2	104
0.05M TBS with 30mg/mL BSA, pH 7.2	112
0.05M PBS, pH 7.2	97
MOP, pH 4.5	50
Citrate Phosphate with 1mg/mL BSA, pH 6.0	102
0.1M Acetate with 10mg/mL BSA, 1% Triton, pH 5.0	97
Carbonate, pH 9.5	85

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 CHO HCPs may read less than the 250ng/mL kit standard. Samples yielding signals above the 250ng/mL standard, or suspected of having concentrations in excess of 10,000ng/mL should be assayed diluted.

Report Date

This report was generated December 7, 1999.