

CHO-CM HCP ELISA Assay

Validation Summary Catalog # CM015

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 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 CHO proteins or have very low levels (<2ng/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 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-CM HCP ELISA Kit, Lots #27041, 26021 & 28050	Cat #CM015
The protocol as defined in the kit insert was used in this validation.	
Data References: Raw data for these experiments are recorded in Cygnus Notebook.	#1-CHO-CM

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. 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
10	1.6	14.4
10	5.9	7.2
10	60.5	5.5

Inter-assay:

# of assays	Mean ng/mL	%CV
5	1.8	13.0
5	6.4	7.7
5	69.2	6.2

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 ~ 660pg/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

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 CHO HCPs recovered from protein free media conditioned with a K1 strain of CHO. Other strains of CHO have been shown to give a high degree of homology with these antibodies, however the user may want to perform a Western Blot with the antibodies used in this kit (M0016-AF) to establish that they are sufficiently reactive to HCPs found from their process and product. Cygnus sells a

Western Blot kit, Catalog #CM060, which utilizes the same antibodies as in this ELISA kit. Cross-reactivity has not been extensively evaluated with this kit. 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

To obtain additional product information contact Cygnus Technologies:

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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 8, and 75ng/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	101
0.05M TBS with 3mg/mL human IgG, pH 7.2	93
0.05M PBS with 10mg/mL BSA, pH 7.2	96
Citrate Phosphate with 1mg/mL human IgG, pH 6.0	102
0.1M Acetate with 10mg/mL human IgG, 1% Triton, pH 5.0	97

Hook Capacity

Very high concentrations of HCPs, >200ng/mL, were evaluated for the hook effect. At concentrations exceeding 20,000ng/mL, the apparent concentration of CHO HCPs may read less than the 200ng/mL kit standard. Samples yielding signals above the 200ng/mL standard, or suspected of having concentrations in excess of 20,000ng/mL should be assayed diluted.

Report Date

This report was generated April 3, 2001.