E. coli HCP ELISA Assay

Validation Summary
Catalog # F410

Summary and Explanation

The data summarized below was generated by Cygnus Technologies to establish the performance parameters and validity of this kit to measure *E. coli* 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 experiments discussed below to validate this kit for use with their product. (1) Each user should perform a western blot using the same antibody (AP117) used in this kit to demonstrate that the antibody reacts with the majority of proteins separated by SDS/PAGE. (2) Each user should perform intra and inter assay precision experiments to establish their procedural proficiency. (3) Each user should perform spike recovery experiments using their test sample matrices. Such a study can be performed by adding known amounts of the 100ng/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 *E. coli* proteins or have very low levels (<3ng/mL) determined prior to adding the 100ng/mL standard. Such an experiment will establish the degree of sample matrix interference in the recovery of HCPs. (4) 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 the approved diluent for this assay, Catalog # I028, 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	
Goat anti-*E.coli*:HRP Conjugate, Lots 2535A & 2535B	Cat #F411
Microtiter coated plate, Lots 1345, 1645, 2245	Cat #F412
E.coli HCP Standards, Lot 1545	Cat #F413
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.	#EC:HRP 2005 Pages 1-12
The assay method validated herein uses materials and Standard Operating Procedures (SOPs) common to the production of kits for many other analytes routinely manufactured by Cygnus Technologies. These SOPS and kits are time tested over several years, well characterized, and validated. Cygnus conducts its R&D and manufacturing operations according to the essentials of GLP and cGMP regulations and guidelines.	

Antibody Development & Characterization

The antibody used in this kit was generated against a number of commonly used laboratory strains and a non-pathogenic clinical isolate. These strains are as follows: BL21, DH5 alpha, and from Invitrogen: strains TOP 10F, JM109, HB101, and MC1061. Western blot analysis and ELISA dilutional analysis of these and other individual strains, indicates that the vast majority of ECPs are antigenically conserved among all strains of *E.coli.* Thus this kit should be of utility for strains other than those specifically used above. It is recommended that each user of this kit verify by western blotting and ELISA, that the kit antibody reacts with the majority of their ECPs.

Sensitivity

Limit of Detection (LOD) - The *E. coli* HCP concentration corresponding to an OD signal 2 standard deviations above the mean of the zero standard is defined as the 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 200 pg/mL.

Limit of Quantitation (LOQ) - 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 levels and then interpolating that concentration which corresponds to a 20% CV. Because this assay shows very excellent precision, the %CVs for all standards were all above 20%. In such cases the default for claimed LOQ is the lowest dosed standard. We therefore very conservatively claim the LOQ as <1ng/mL but statistical evidence suggests that is in fact lower.

Specificity

The following strains were used to generate antibodies and have expressed a high degree of homology as determined by Western Blot analysis: BL21, DH5 alpha, JM109, HB101, TOP10F, and MC1061. Several other strains of *E.coli* such as K12 have been reported to have very similar high degrees of homology when compared to the immunization strains. Each user should evaluate the suitability of these antibodies for detection of their process HCPs by both Western Blot and this ELISA and comparing results to protein bands detected by a sensitive PAGE protein staining method, such as silver staining. Other *Enterobacteriacea* species have not been tested but may cross-react with these antibodies. Certain biological reagents may have been contaminated with *E.coli* or other *Enterobacteriacea* species during their production or storage. For example, Cygnus Technologies has detected *E. coli* HCP activity in many commercial preparations of bovine serum albumin and other protein preparations. These are presumably carryover trace contaminates of HCPs since these materials were negative by culture. Such HCP contamination of reagents can lead to apparent elevation of HCP by this assay and could thus be erroneously attributed to the user's product and process. Therefore, it is advisable that the user test such raw materials for HCP activity.

Precision

Precision is defined as the percent coefficient of variation (%CV). This is calculated by dividing the standard deviation by the mean for a number of replicate determinations of three different control samples in the low, mid and high concentration range of the assay. Both within (intra-assay) and between (inter-assay) precision were determined. 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. For labs having difficulty in routinely achieving these specifications it is suggested they assay all samples at least in triplicate to better identify statistical outliers.

Intra-assay:

# of tests	Mean ng/mL	%CV	Design Goal Specification
20	2.84	3.3	<20%
20	11.78	1.9	<10%
20	39.14	2.7	<10%

Inter-asay:

# of assays	Mean ng/mL	%CV	Design Goal Specification
6	3.03	4.2	<20%
6	12.20	1.6	<10%
6	40.69	4.1	<10%

Recovery/Matrix Interference

The same *E. coli* HCP preparation used for the standards was spiked into various "sample buffers" to demonstrate the potential for matrix interference. HCPs were added at 20ng/mL and tested in duplicate. 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). Each user is encouraged to test their sample matrices for recovery in a similar experiment. When diluting samples the ideal diluent is the same material as is used to prepare the kit standards, Catalog #I028. If you chose to use some other diluent you must validate that it yields a background OD the same as the kit zero standard, and that it gives a dilution concentration response curve that is parallel to the kit standards.

Sample Buffer Matrix	*E.coli* added ng/mL	*E.coli* recovered ng/mL	% Recovery (assayed/ added x100)
0.05M PBS with 1 mg/mL BSA, pH 7.0	20	18.6	93
0.05M TBS with 8 mg/mL BSA, pH 7.2	20	19.7	99
0.05M TBS with 0.1mg/mL BSA, pH 8.5	20	19.8	99
0.02M Acetate/NaCl + 0.1mg/mL BSA, pH 5.5	20	15.8	79
0.02M Acetate with 1% Triton X-100	20	24.1	120
0.05M TBS + 1mg/mL BSA, 0.01% Tween 20, pH 7.0	20	15.0	75
0.05M TBS + 1mg/mL BSA, 0.1% Tween 20, pH 7.0	20	12.2	61
0.05M TBS + 1mg/mL BSA, 0.1% Triton, pH 7.0	20	12.3	62

Hook Capacity

Very high concentrations of *E. coli* HCPs were evaluated for the hook effect. At concentrations exceeding 200µg/mL the apparent concentration of *E. coli* HCPs may read less than the 100ng/mL kit standard. Samples yielding signals above the 100ng/mL standard or suspected of having concentrations in excess of 200µg/mL should be assayed diluted.

Reagent Stability

Stability of this kit and its components was established by both real time and accelerated (storage at 37°C) conditions. Stability continues to be monitored real time as part of our routine QC/QA SOPs. When stored as specified on the labels all components are stable until the kit expiration date.

Report Date

This report was generated April 24, 2005.

Company Information

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