



Cygnus Technologies, Inc.

4705 Southport Supply Road SE, Suite 208
Southport, NC 28461 USA
Tel: 910-454-9442 FAX: 910-454-9443
Email: cygnustec@aol.com
Web: www.cygnustechnologies.com

***L. lactis* HCP ELISA kit
Catalog # F490
Validation Summary
Report dated January 8, 2008**

The data summarized below was generated by *Cygnus Technologies* to establish the performance parameters and validity of this kit to measure *L. lactis* 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 a western blot or equivalent method using the same antibody used in this kit to demonstrate that the antibody reacts with the majority of proteins fractionated by SDS/PAGE or liquid chromatography. (2) Each user should perform intra and inter assay precision experiments to establish their procedural proficiency. (3) Each user should perform 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 *L. lactis* 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 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: Goat anti-*L. lactis*:HRP Conjugate lots 107-97, 107-107, & 107-117
Coated plate lots 23107 & 25107
Standards lot 26107

Methods: The assay protocol as described in the kit insert was used in this validation.

Data References: Raw data for these experiments are recorded in Notebook # LL1-07 pp 1-27.

Antibody Development and Characterization: The antibody used in this kit was generated against HCPs extracted from spent culture media for a null cell line. This kit should be of utility for other strains of *L. lactis* however 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 HCPs.

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	2.694	4.8	<20%	10	2.988	4.9	<20%
20	11.722	2.7	<10%	10	11.846	2.3	<10%
20	40.549	2.6	<10%	10	39.738	2.2	<10%

Recovery/Matrix Interference: The same *L. lactis* HCP preparation used for the standards was spiked into various “sample buffers” from a multi-step purification process to demonstrate the potential for matrix interference. HCPs were added at 50ng/mL and tested in duplicate. In all cases the zero for each sample buffer matrix 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. 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. Samples containing endogenous process derived levels of HCPs must first be diluted into the assay analytical range prior to performing the spike recovery experiment. Ideally the levels of endogenous HCP should be low so as not to significantly affect the determination of the spike levels. When diluting samples the ideal diluent is the same material as is used to prepare the kit standards. Cygnus Technologies sells the recommend diluent for this purpose as Catalog # I-028. 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	<i>L. lactis</i> Added ng/mL	<i>L. lactis</i> Recovered ng/mL	% Recovery (assayed/added x100)
Culture media	50	51.65	103.3
Diafiltrate of Culture media into PBS	50	53.60	107.2
1 st chromatographic purification step	50	48.70	97.4
2 nd chromatographic purification step	50	50.25	100.5
Final product formulation buffer	50	49.35	98.7

Sample Dilutional Linearity: Actual in-process and final product samples from a recombinant protein expressed in *L. lactis* were subjected to dilutional linearity analysis. In this experiment we were able to obtain a minimum required dilution (MRD) for all sample types within the analytical range of the assay and at which subsequent dilution corrected values remained essentially the same. This is a critical experiment in establishing that the kit antibodies are at excess for the types of HCPs in a given sample and that nothing in the sample matrix inhibits the ability of the assay to detect HCP. Each user must validate that their samples with endogenous levels of HCPs demonstrate acceptable dilutional linearity and yield an MRD defined as “dilution corrected analyte concentrations that vary no more than 80% to 120% between subsequent doubling dilutions”.



HCP Levels in Real Process Samples

Sample Type	MRD	Endogenous Dilution corrected HCP concentration
Culture media	1:160,000	10mg/mL
1 st chromatographic purification step	1:20,000	452µg/mL
2 nd chromatographic purification step	1:2000	57µg/mL
Final product	1:20	2µg/mL

Sensitivity, LOD & LOQ: The *L. lactis* 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 20 replicates of the zero standard. The mean signal of the zero standard plus 2 SD yielded a LOD concentration of 123 pg/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 thus extrapolated to a concentration of <500 pg/mL.

Specificity: A single null cell strain of *L. lactis* was used to generate the antisera and to make assay standards. Other strains of *L. lactis* are likely to show a high degree of conservation among the majority of HCPs. Each user should evaluate the suitability of these antibodies for detection of their process HCPs by both Western Blot or some other equivalent protein fractionation method such as liquid chromatography and this ELISA and comparing results to protein bands detected by a sensitive total protein method such as silver staining. A very limited number of other bacterial species have been tested. *E. coli* and *Pseudomonas fluorescens* were found not to cross react in this assay. It is advisable that the user test such raw materials for HCP activity.

Hook Capacity: Very high concentrations of *L. lactis* were evaluated for the hook effect. At concentrations exceeding 250 µg/mL, the apparent concentration of *L. lactis* may read less than the 100ng/mL standard. Samples yielding signals above the 100ng/mL standard or suspected of having concentrations in excess of 250 µg/mL should be assayed diluted.

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 for the kit expiration date.