



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

***Saccharomyces cerevisiae* HCP ELISA kit**

Catalog # F135

Validation Summary

Report dated Feb. 14, 2001

The data summarized below was generated by *Cygnus Technologies* to establish the performance parameters and validity of this kit to measure *Saccharomyces cerevisiae* 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 to be tested. Ideally these test sample matrices should be devoid of any *Saccharomyces cerevisiae* proteins or have very low levels (<4ng/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 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 # 28120.

Methods: Assay protocol as specified in kit directions insert.

Data References: Raw data for these experiments are recorded in Notebook #SC-01.

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
10	1.6	15.4	<20%	5	1.9	11.1	<20%
10	8.5	2.6	<10%	5	8.3	4.3	<10%
10	75.3	1.8	<10%	5	74.2	5.7	<10%

Sensitivity: The *Saccharomyces cerevisiae* 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 0.5ng/mL. The limit of quantitation (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 points and then interpolating that concentration which corresponds to a 20% CV. The LOQ was 0.9ng/mL.

Specificity: Normal mouse and normal human sera were tested for cross-reactivity at 1mg/mL and at 10µg/mL, and found to be non-reactive in this assay. Solubilized cell proteins from the yeast *Pichia pastoris*, the bacteria *E. coli*, and Chinese Hamster Ovary cells were tested at 1, 75, and 250ng/mL, and found not to cross react in this assay. Each user should determine if other known constituents in their samples are cross reactive or interfere in any way at their typical concentrations.

Hook Capacity: Very high concentrations of HCPs (>200ng/mL) were evaluated for the hook effect. At concentrations exceeding 50µg/mL, the apparent concentration of *Saccharomyces cerevisiae* HCPs may read less than the 200ng/mL standard. Samples yielding signals above the 200ng/mL standard or suspected of having concentrations in excess of 50µg/mL should be assayed diluted.