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## ***Pichia pastoris* HCP ELISA kit**

### **Catalog # F140**

### **Validation Summary**

**Report dated Dec 7, 1999**

The data summarized below was generated by *Cygnus Technologies* to establish the performance parameters and validity of this kit to measure *Pichia pastoris* 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 *Pichia pastoris* 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 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 # 4119, Exp. Date 2/11/00 used for all studies.

**Methods:** Assay protocol as specified in kit Directions Insert revision #1-00.

**Data References:** Raw data for these experiments are recorded in Notebook #PP-10, pages 16-27.

**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	9.3	7.6	<12%	5	9.9	3.4	<15%
20	41.4	2.7	<10%	5	38.3	7.1	<10%
20	134.2	6.6	<10%	5	142.2	4.0	<10%

**Recovery/Matrix Interference:** The same *Pichia pastoris* HCP preparation used for the standards was spiked into various “sample buffers” to demonstrate the potential for matrix interference. HCPs were added at 10, 33, and 100 ng/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 0 ng/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	Average % Recovery (assayed/added x100)
0.05M TBS with 10mg/mL BSA, pH 7.2	103
0.05M TBS with 30 mg/mL BSA. pH 7.2	113
0.05M PBS pH 7.2	91
MOP, pH 4.5	47
Citrate/Phosphate with 1 mg/mL BSA, pH 6.0	100
0.05M TBS pH 7.2	91
0.1M Acetate, 10mg/mL BSA, 1% Triton, pH5.0	97
Carbonate, pH 9.5	85
0.05 M Tris, 10mg/mL BSA, pH 8.5	97

**Sensitivity:** The *Pichia pastoris* 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 ~ 250pg/mL. The LOD of the rapid protocol was 900pg/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 350pg/mL for the standard protocol and 1.0ng/mL for the rapid protocol.

**Specificity:** Cross reactivity has not been extensively evaluated with this kit. Western Blot of another yeast, *Saccharomyces cerevisiae*, with the antibodies used in this kit did show some binding to approximately 5 % of the total protein bands. On a weight to weight basis, the *Saccharomyces cerevisiae* cross reactivity was less than 1.3% in this ELISA kit. Each user should determine if known constituents in their samples can cause cross reactivity.

**Hook Capacity:** Very high concentrations of HCPs (>250ng/mL) were evaluated for the hook effect. At concentrations exceeding 32,000ng/mL, the apparent concentration of *Pichia pastoris* HCPs may read less than the 250ng/mL standard. Samples yielding signals above the 250ng/mL standard or suspected of having concentrations in excess of 32,000ng /mL should be assayed diluted.