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Ultra Sensitive Insulin ELISA kit
Catalog # F280
Validation Summary
Report dated August. 24, 2003

The data summarized below was generated by *Cygnus Technologies* to establish the performance parameters and validity of this kit to measure bovine, porcine or human insulins. This data is intended to supplement and not replace user generated validation data. This 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 1250pg/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 Insulin or have very low levels (<25pg/mL) determined prior to adding the 1250pg/mL standard. Such an experiment will establish the degree of sample matrix interference in the recovery of insulin. (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 insulin. 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 insulin in the “Hook Region” of the concentration response curve.

Materials: Anti Insulin Coated Strip Lot #'s 173 and 1583.
Insulin Standards Lot # 1183
Conjugate Stock Lot # 415-73

Methods: Assay protocol as specified in kit Product Insert.

Data References: Raw data for these experiments are recorded in *Cygnus Technologies, Inc.* Notebook # USInsulin-001.

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
12	48.0	10.2	<12%	5	50.3	6.3	<15%
12	189.4	3.0	<10%	5	196.8	3.5	<10%
12	932.1	2.5	<10%	5	925.7	1.7	<10%

Recovery/Matrix Interference: The same Insulin preparation used for the standards was spiked into various “sample buffers” to demonstrate the potential for matrix interference. Insulin were added at 75, 200, and 1000pg/mL and tested in duplicate. The average % recovery is reported in the last column. Acceptable recovery is specified as plus or minus 20% of the added Insulin value. These data serve as examples of certain buffers or buffer components which may or may not give matrix interference. Matrix interference can be either positive (false increase in insulin) or negative (false decrease in Insulin). 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)
Citrate/Sucrose/Arginine/Tween 80 with 1mg/mL Human IgG, pH 7.0	98
Tris/Phosphate/Sucrose with 1mg/mL BSA, pH 7.5	87
Tris Buffer Saline with 4mg/mL human IgG, pH 7.2	70

Sensitivity: The Insulin 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 of ~ 6.0pg/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 25pg/mL.

Specificity: The antibodies used in this kit substantially cross-react (~100 %) with insulin from human (natural and recombinant), bovine and porcine. Cross reactivity with insulin from other species has not been extensively investigated. Rat and Mouse insulins have been reported to cross-react in the range of 50 to 70%. This kit can be used to quantitate rat and mouse insulin provided the laboratory has its own standards or can apply the appropriate cross reactivity correction factor.

Cross Reactant	% Cross Reactivity
Bovine Insulin	100
Porcine Insulin	96
Human Insulin, natural	101
Human Insulin, recombinant	104

Hook Capacity: Very high concentrations of Insulin (up to 2.0µg/mL) were evaluated for the hook effect. At concentrations exceeding 2.0µg/mL, the apparent concentration of Insulin may read less than the 1250pg/mL standard. Samples yielding signals above the 1250pg/mL standard or suspected of having concentrations in excess of 2.0µg /mL should be assayed diluted.