

Insulin ELISA Assay

Validation Summary Catalog # F040

Summary and Explanation

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. 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 20ng/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 (<0.25ng/mL) determined prior to adding the 20ng/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 are to be serially diluted with Sample Diluent Buffer, Catalog # I028 the approved diluent for this assay, 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 insulin in the "Hook Region" of the concentration response curve.

Materials & Methods Used

Materials	
Insulin ELISA Kit, Lot #2196 & 7067	Cat #F040
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.	#1- Insulin, 1966 Pages 1-37

Precision

Precision is defined as the percent coefficient of variation (%CV). This is calculated by dividing the standard deviation by the mean value for a number of replicate determinations of two different control samples in the low 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:

# of tests	Mean ng/mL	%CV	Design Goal Specification
12	0.80	6.7	<10%
12	8.11	5.8	<10%

Inter-assay:

# of assays	Mean ng/mL	%CV	Design Goal Specification
5	0.81	8.4	<12%
5	8.0	5.9	<12%

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 10 replicates of the zero standard. The mean signal of the zero standard plus 2 SD yielded a LOD of ~ 125pg/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 155pg/mL. Unless your laboratory has determined such precision profile, we recommend a more conservative claim to LOQ as the first dose standard or LOQ of 250pg/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

Spike & Recovery

Bovine insulin was spiked into MEM culture media. Recovery specification is 90% - 110%, between 0.5 to 8ng/mL. Results are listed below.

Insulin Added ng/mL	Insulin Recovered ng/mL	% Recovery (assayed/added x100)
0.5	0.48	96
2.0	1.91	95
8.0	7.66	96

Hook Capacity

Very high concentrations of insulin, up to 6.0µg/mL, were evaluated for the hook effect. At concentrations exceeding 6.0µg/mL, the apparent concentration of insulin may read less than the 20ng/mL kit standard. Samples yielding signals above the 20ng/mL standard, or suspected of having concentrations in excess of 6.0µg/mL should be assayed diluted.

Report Date

This report was generated March 2, 2002.

Company Information

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