



Cygnus Technologies, Inc.

4705 Southport Supply Road, Suite 208

Southport, NC 28461 USA

Tel: 910-454-9442 FAX: 910-454-9443

Email: cygnustec@aol.com

Web: www.cygnustechnologies.com

Human Serum Albumin ELISA kit

Catalog # F055

Validation Summary

Report dated June 20, 1999

The data summarized below was generated by *Cygnus Technologies* to establish the performance parameters and validity of this kit to measure HSA. 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 HSA or have very low levels (< 0.5ng/mL) determined prior to adding the 20ng/mL standard. Such an experiment will establish the degree of sample matrix interference in the recovery of HSA (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 HSA. 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 HSA in the "Hook Region" of the concentration response curve.

Materials: Kit Lot 71097

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

Data References: Raw data for these experiments are recorded in Notebooks #1-HSA pages 1-14.

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				Inter-assay			
# of tests	Mean ng/mL	%CV	Design Goal Specification	# of assays	Mean ng/mL	%CV	Design Goal Specification
20	2.1	7.0	<10%	5	2.6	7.6	<12%
20	19.5	4.4	<8%	5	19.8	5.1	<10%

Sensitivity: The HSA 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 280pg/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 150pg/mL.

Specificity: In sandwich ELISA, cross reactivity can manifest itself either as a false increase in HSA (positive cross reactivity) or as a false decrease in HSA (negative cross reactivity) when HSA present in the sample competes with the cross reactant for the kit antibodies. The following materials were tested for cross reactivity at the concentrations indicated both in the absence of HSA and in the presence of 5ng/mL HSA. None of these materials were found to yield any statistically significant false increase or decrease in apparent HSA concentrations. While no cross reactivity was detected in any of the substances tested it is recommended that each user test known materials in their sample matrices for cross reactivity in a similar experiment.

Materials Not Cross Reactive for HSA

Substance	Concentration Tested	% Cross Reactivity
BSA	2 mg/mL	0
Mouse serum neat	“	0
Mouse serum, 1:1000	“	0

Hook Capacity: Very high concentrations HSA were evaluated for the hook effect. At concentrations exceeding 20,000ng/mL, the apparent concentration of HSA may read less than the 20ng/mL standard. Samples yielding signals above the 20ng/mL standard or suspected of having concentrations in excess of 20,000ng/mL should be assayed diluted.