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BSA ELISA kit
Catalog # F030
Validation Summary
Report dated Oct. 12, 1997

The data summarized below was generated by *Cygnus Technologies* to establish the performance parameters and validity of this kit to measure Bovine Serum Albumin (BSA). 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 32ng/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 BSA or have very low levels (< 0.5 ng/mL) determined prior to adding the 32ng/mL standard. Such an experiment will establish the degree of sample matrix interference in the recovery of BSA (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 BSA. 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 BSA in the "Hook Region" of the concentration response curve.

Materials: Kit Lot 8266

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-BSA pages 1-52.

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
12	2.0	7.4	<10%	5	2.1	8.1	<12%
20	12.2	5.3	<8%	5	12.2	6.7	<10%

Recovery/Matrix Interference:

The same BSA preparation used for the standards was spiked into various “sample buffers” to demonstrate the potential for matrix interference. BSA was added at 15 ng/mL and tested in duplicate. 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 BSA. Acceptable recovery is specified as plus or minus 20% of the added BSA 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 BSA) or negative (false decrease in BSA). Each user is encouraged to test their sample matrices for recovery in a similar experiment.

Sample Buffer Matrix	BSA Added ng/mL	BSA Recovered ng/mL	% Recovery (assayed/added x100)
0.05M TBS with 10% mouse serum, pH 7.2	15	16.1	107
0.05M TBS with 10% goat serum, pH 7.2	15	16.3	109
0.05M TBS with 10mg/mL porcine gelatin, pH 7.2	15	13.3	89
0.05M TBS with 10% rabbit serum, pH 7.2	15	8.7	58
0.05M PBS with 5µM EDTA, pH 7.4	15	13.1	87
Glycine/HCl pH 2.4	15	12.0	80
0.05 M Carbonate, pH 9.5	15	12.3	82
Tris Glycine with 1% SDS	15	7.1	47
0.05M Tris pH 8.5	15	14.4	96
0.1M Acetate, 1% Triton, pH4.5	15	9.8	65
0.05M TBS with 1% fish Gelatin, pH7.0	15	15.0	100

Sensitivity: The BSA 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 150 pg/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 250 pg/mL.

Specificity: In sandwich ELISA, cross reactivity can manifest itself either as a false increase in BSA (positive cross reactivity) or as a false decrease in BSA (negative cross reactivity) when BSA 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 BSA and in the presence of 15 ng/mL BSA. None of these materials were found to yield any statistically significant false increase or decrease in apparent BSA 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 BSA

Substance	**Concentration Tested**
Human albumin	10 mg/mL
Goat serum	10%
Mouse serum	10%
Rabbit serum	10%
Porcine gelatin	10mg/mL
Fish gelatin	1%

Hook Capacity: Very high concentrations of BSA were evaluated for the hook effect. At concentrations exceeding 10,000 ng/mL, the apparent concentration of BSA may read less than the 32 ng/mL standard. Samples yielding signals above the 32 ng/mL standard or suspected of having concentrations in excess of 10,000 ng /mL should be assayed diluted.