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**Protein A ELISA kit
Catalog # F050
Validation Summary
Report dated Nov., 1998**

The data summarized below was generated by *Cygnus Technologies* to establish the performance parameters and validity of this kit to measure Protein A. 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 16ng/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 Protein A or have very low levels (< 0.25ng/mL), determined prior to adding the 16ng/mL standard. Such an experiment will establish the degree of sample matrix interference in the recovery of Protein A. (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 Protein A. Such samples are to 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 Protein A in the "Hook Region" of the concentration response curve.

Materials: Kit Lot 3118

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 #2 pages 1-40.

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	1.0	6.3	<10%	5	1.1	8.3	<12%
20	4.2	5.4	<8%	5	4.2	6.5	<10%

Recovery/Matrix Interference: The same Protein A preparation used for the standards was spiked into various “sample buffers” to demonstrate the potential for matrix interference. Protein A was added at 4ng/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 0ng/mL of Protein A. Acceptable recovery is specified as plus or minus 20% of the added Protein A 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 Protein A or negative (false decrease) in Protein A. Each user is encouraged to test their sample matrices for recovery in a similar experiment. Immunoglobulin in your samples that is bound to Protein A may interfere with the ability of the antibodies used in this kit to detect Protein A, resulting in significant under-recovery of true Protein A values. See the “Specificity” section below. If you experience unacceptable recovery of Protein A in the presence of your sample antibody, contact *Cygnus Technologies* technical services for advice on how to solve this problem. Some natural human IgGs and rabbit IgGs have been shown to yield low recovery of spiked Protein A. *Cygnus Technologies* manufactures another Protein A-h ELISA kit, Catalog # F050H, that is designed to eliminate under-recovery from interfering antibodies.

Sample Buffer Matrix	Protein A Added ng/mL	Protein A Recovered ng/mL	% Recovery (assayed/added x100)
0.05M TBS with 10% mouse serum, pH 7.2	4	3.9	98
0.05M TBS with 10% human serum, pH 7.2	4	3.3	83
0.05M PBS, 5 mg/ml mouse IgG, 5µM EDTA, pH 7.4	4	3.6	90
Glycine/HCl pH 2.4 with 5mg/mL mouse IgG	4	2.9	73
0.05 M Carbonate, 5mg/mL mouse IgG, pH 9.5	4	3.2	80
Tris Glycine with 1% SDS	4	2.1	53
0.05M Tris pH 8.5, with 2mg/mL recombinant human IgG	4	3.7	93
0.1M Acetate, 1% Triton, pH4.5	4	3.3	83
0.05M TBS with 1mg/mL of a human mouse chimeric antibody, pH7.0	4	3.7	93

Sensitivity: The Protein A 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 0.12ng/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 0.20ng/mL.

Specificity: Natural and recombinant Protein A to include truncated fragments of Protein A were tested for cross reactivity. On a molar basis, all forms of Protein A reacted essentially equally with recovery between 90 to 100%. However, you may want to test your source of Protein A for recovery to ensure accurate quantitation by this kit.

When present in the sample to be tested, most humanized antibodies or human antibodies produced by cell culture in CHO or hybridoma cell lines will yield accurate recovery of Protein A. However, some human and rabbit IgGs have been shown to inhibit the ability of the kit anti-Protein A antibodies to bind to Protein A, resulting in an under-recovery of true Protein A contamination. Your product antibodies should be evaluated for this negative interference before reporting results. If you encounter product antibody interference contact *Cygnus Technologies* for advice on how to solve this problem.

Hook Capacity: Very high concentrations of Protein A were evaluated for the hook effect. At concentrations exceeding 20,000ng/mL, the apparent concentration of Protein A may read less than the 16ng/mL standard. Samples yielding signals above the 16ng/mL standard or suspected of having concentrations in excess of 20,000ng/mL should be assayed diluted.