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**Human Transferrin ELISA kit
Catalog # F035N
Validation Summary**

The data summarized below was generated by *Cygnus Technologies* to establish the performance parameters and validity of this kit to measure human transferrin. 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 8ng/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 human transferrin or have very low levels (<0.5ng/mL) determined prior to adding the 8ng/mL standard. Such an experiment will establish the degree of sample matrix interference in the recovery of human transferrin (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 human transferrin. 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 human transferrin in the “Hook Region” of the concentration response curve.

Materials: Kit Lots 1120, 16041

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-human transferrin pages 40-49.

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	0.48	7.2	<10%	5	0.47	8.5	<12%
20	4.04	3.3	<8%	5	4.06	5.6	<10%

Sensitivity: The human transferrin 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 50pg/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 125pg/mL.

Specificity: Human transferrin from three different commercial sources was tested and found to react essentially 100% with the standards used in this kit. Holo transferrin is 100% reactive on a molecular weight basis. Bovine transferrin was tested at 2ng/mL and gave no apparent reactivity in this assay. There was also no significant reactivity to horse, sheep, dog, rat or pig serum.

Hook Capacity: Very high concentrations human transferrin were evaluated for the hook effect. At concentrations exceeding 200µg/mL, the apparent concentration of human transferrin may read less than the 8ng/mL standard. Samples yielding signals above the 8ng/mL standard or suspected of having concentrations in excess of 200µg/mL should be assayed diluted.