

Human Transferrin ELISA Assay

Validation Summary Catalog # F035N

Summary and Explanation

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 more comprehensive user generated validation. The data is representative of what a laboratory can expect to achieve for precision and sensitivity 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 spike 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 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. In addition, good dilutional linearity demonstrates specificity and freedom from some types of sample matrix interference.

Materials & Methods Used

Materials	
Human Transferrin ELISA kit, Lot #1120 & 16041	Cat #F035N
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-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:

# of tests	Mean ng/mL	%CV	Design Goal Specification
20	0.48	7.2	<10%
20	4.04	3.0	<8%

Inter-assay:

# of assays	Mean ng/mL	%CV	Design Goal Specification
5	0.47	8.5	<12%
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 200 μ g/mL, the apparent concentration of human transferrin may read less than the 8ng/mL kit standard. Samples yielding signals above the 8ng/mL standard or suspected of having concentrations in excess of 200 μ g/mL should be assayed diluted.

Report Date

This report was generated April 16, 2001.

Company Information

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