



Product Manual

Transferrin ELISA kit

Catalog #: ENZ-KIT143

96 Well Kit



Product Manual

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Please read
entire booklet
before
proceeding with
the assay.



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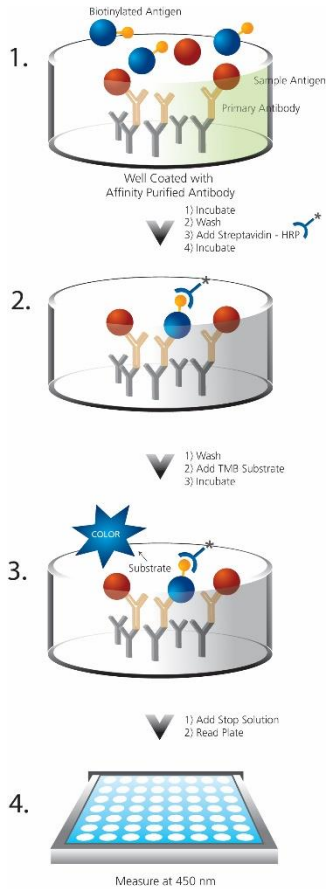
INTRODUCTION

The Enzo Transferrin ELISA kit is a complete kit for the quantitative determination of transferrin in human and canine sample matrices. This competitive, ultra-sensitive ELISA has a time-to-answer of 2 hours. Please read the complete kit insert before performing this assay.

Human serum transferrin, one of the members of the family of transferrin proteins, is an 80kDa glycoprotein and is a major carrier of iron in blood¹. Also included in this family of proteins are: i) lactoferrin, which is the iron-binding protein found in human milk and ii) ovotransferrin, which is the iron-binding protein isolated from avian egg white². Transferrin binds iron, forming a transferrin-iron complex. When this complex encounters a transferrin receptor on the surface of the cell, it binds and is transported into the cytoplasm. Once inside the cell, the iron is released and the iron-free transferrin, now referred to as “apotransferrin”, is transported back to the cell surface. Each molecule of transferrin can bind up to two atoms of iron to form reddish protein-iron complexes³. Transferrin allows for the transport of the ferric iron (Fe^{3+}) from the intestine, reticuloendothelial system and liver parenchymal cells to all cells in the body⁴.

Measurements of serum transferrin has applications in anemia, where medical professionals may check transferrin levels in iron deficiency, as an increase in transferrin levels may be seen in patients with iron deficiency anemia. Additionally, transferrin has a role in iron overload disorders such as hemochromatosis, where transferrin levels would be decreased in plasma and serum⁵. Decreased levels of transferrin can also be detected in patients with liver diseases, such as cirrhosis⁶ and non-alcoholic fatty liver disease⁷.

PRINCIPLE



1. Samples or standards are added to wells coated with a goat anti-rabbit IgG antibody. A solution of biotinylated transferrin tracer and a polyclonal antibody to transferrin are both added to the wells.
2. The plate is incubated. During this incubation, the antibody binds the transferrin in the sample or tracer in a competitive manner.
3. The plate is washed, leaving only bound transferrin on the plate. A solution of Horseradish Peroxidase conjugated Streptavidin (SA-HRP) is added to all wells and the plate is incubated.
4. The plate is washed to remove excess conjugate. TMB substrate is added to the wells and the plate is incubated.
5. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450nm. The amount of signal is indirectly proportional to the level of transferrin in the sample.



Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

MATERIALS SUPPLIED

1. **Goat anti-Rabbit IgG Microtiter Plate, One Plate of 96 Wells**
Catalog No. 80-0060
A plate using break-apart strips coated with a goat anti-rabbit IgG antibody.
2. **Assay Buffer 13, 1x, 25 mL**
Catalog No. 80-2305
Tris buffered saline containing proteins and detergents.
3. **Transferrin Antibody, Lyophilized**
Catalog No. 80-2688
Lyophilized polyclonal antibody specific to transferrin.
4. **Antibody Diluent, 6 mL**
Catalog No. 80-2680
Buffer for dilution of reconstituted transferrin antibody.
5. **Transferrin Tracer, Lyophilized**
Catalog No. 80-2687
Lyophilized biotinylated transferrin tracer.
6. **Tracer Diluent, 6mL**
Catalog No. 80-2681
Buffer for dilution of reconstituted transferrin tracer.
7. **Wash Buffer Concentrate, 20x, 100 mL**
Catalog No. 80-1287
Tris buffered saline containing detergents.
8. **Transferrin Standard, 200 μ L**
Catalog No. 80-2686
One vial containing 50 μ g/mL transferrin.
9. **SA-HRP Conjugate, 20 mL**
Catalog No. 80-2689
A solution of Streptavidin-conjugated Horseradish Peroxidase.
10. **TMB Substrate, 25 mL**
Catalog No. 80-2101
A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide.
11. **Stop Solution 2, 10 mL**
Catalog No. 80-0377
A 1N solution of hydrochloric acid in water.
12. **Transferrin Assay Layout Sheet, 1 each**
Catalog No. 30-0333
13. **Plate Sealer, 3 each**
Catalog No. 30-0012

STORAGE

All of the components of this kit are stable at 4°C except for the standard which must be stored at -20°C until the kit's expiration date.

OTHER MATERIALS NEEDED

1. Deionized or distilled water
2. Precision pipets for volumes between 5 μ L and 1,000 μ L
3. Repeater pipet for dispensing volumes between 50 μ L and 200 μ L
4. Disposable beakers for diluting buffer concentrates.
5. Graduated cylinders
6. A microplate shaker
7. Adsorbent paper for blotting
8. Microplate reader capable of reading at 450 nm



Samples must be stored at or below -20°C to avoid loss of bioactive analyte. Avoid repeated freeze/ thaw cycles.



If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

SAMPLE HANDLING

This assay is suitable for measuring transferrin in human serum and EDTA plasma in addition to canine serum. Prior to sample analysis in the assay, frozen samples should be slowly thawed at room temperature and vortexed to mix.

Human serum and plasma and canine serum samples have been validated for use in this assay (please refer to the Spike and Recovery section on page 7 for detailed data). The physiological levels of transferrin in the neat human serum and plasma samples utilized to validate this assay ranged from ~2-4 mg/mL. As a result, a large dilution is required in this assay. However, due to variation in samples, users *may* need to determine the optimal dilution(s) for their particular experiments.

The following experiments in serum and plasma were utilized to determine the final recommended sample dilutions.

SAMPLE MATRIX PROPERTIES

Linearity

Human serum and EDTA plasma samples with biologically relevant levels of transferrin were diluted 1:10,000 and then serially diluted 1:2 in assay buffer. Additionally, neat canine serum was serially diluted 1:2 in assay buffer. Samples were run in the assay and compared to the standard curve. The results are shown in the table below.

Dilutional Linearity, %		
Dilution	Human serum	Human EDTA plasma
1:10,000	100%	100%
1:20,000	77.8%	106.6%
1:40,000	74.9%	112.8%
1:80,000	60.4%	110.7%

Dilutional Linearity, %	
Dilution	Canine serum
Neat	100%
1:2	108%
1:4	112%
1:8	106%
1:16	102%
1:32	100%

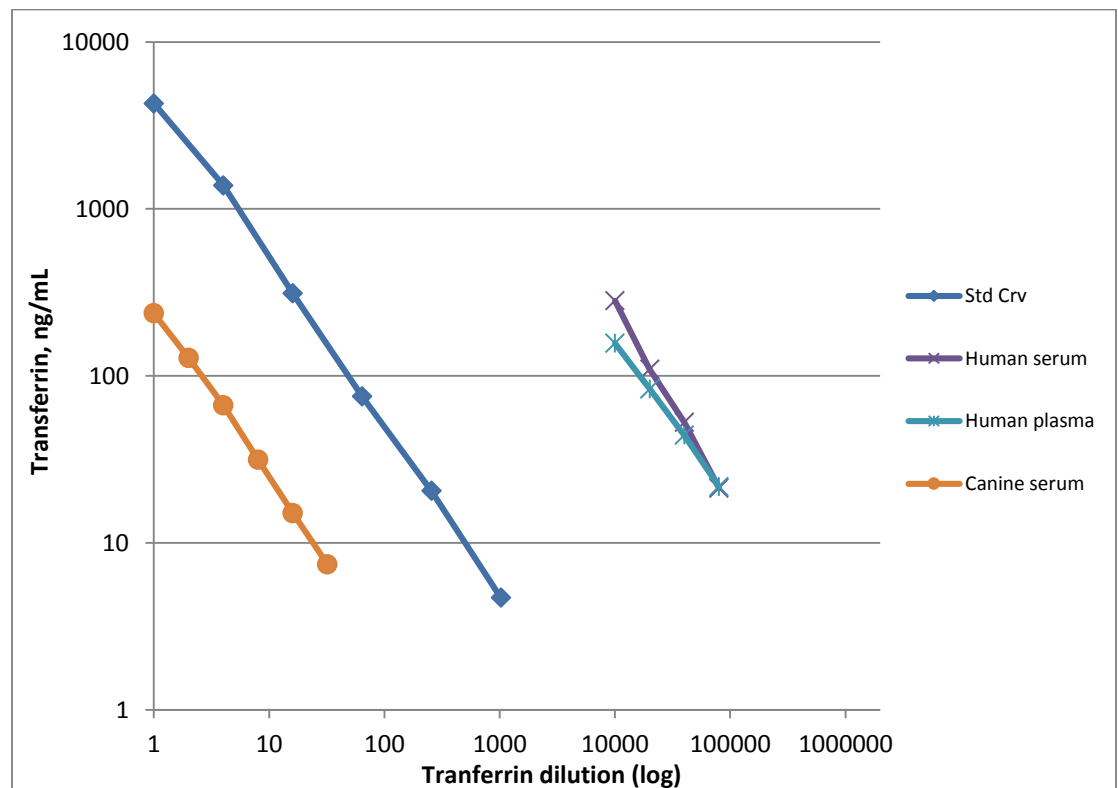
Spike and Recovery

Transferrin was spiked at three concentrations into diluted human serum and EDTA plasma and neat canine serum. Matrix background was subtracted and the recovery was compared to the recovery of identical transferrin spiked into assay buffer. The average percent recovery for each matrix at the minimum recommended dilution (MRD) is indicated below.

Sample	Spike Concentration, ng/mL	% Recovery	Minimum Recommended Dilution
Human Serum	500	106	1:10,000
	75	73	
	10	70	
Human EDTA Plasma	500	107	1:10,000
	75	111	
	20	112	
Canine Serum	1000	106	Neat
	75	150	
	20	175	

Parallelism

To assess parallelism, human serum and EDTA plasma were diluted to the minimum recommended dilution (MRD) and then serially diluted 1:2 in assay buffer. Additionally, neat canine serum was also serially diluted 1:2 in assay buffer. All of the samples were then run in the assay. The transferrin concentration in each sample was assigned using the standard curve. Assigned concentrations were plotted as a function of sample dilution. Parallelism of the curves demonstrates that the antigen binding characteristics are similar enough to allow the accurate determination of native analyte levels in diluted samples of human and canine origin.



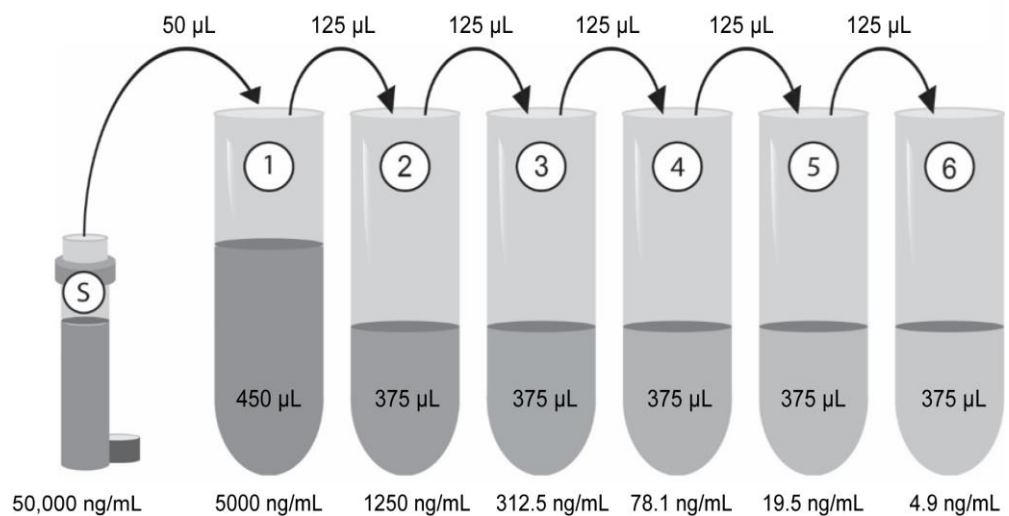
REAGENT PREPARATION

1. Wash Buffer

Prepare the Wash Buffer by diluting 50 mL of the supplied concentrate with 950 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. Transferrin Standard Curve

Allow the transferrin standard stock to warm to room temperature prior to preparation of the diluted standards. Once the dilutions have been prepared, the standard stock and diluted standards can be stored on ice but should be used within **60 minutes** of preparation for optimal performance. All reagents **must** be warmed to room temperature prior to running the assay.



Label six 12x75 mm polypropylene tubes #1 through #6. Add 450µL of Assay Buffer 13 into tube #1. Add 375 µL of Assay Buffer 13 into tube #2 through tube #6. Add 50 µL of 50,000 ng/mL transferrin standard stock to tube #1 and vortex. Add 125 µL of tube #1 into tube #2 and vortex. Add 125 µL of tube #2 to tube #3 and vortex thoroughly. Continue this for tubes #4 through #6.

Diluted standards should not be stored for re-use. Make new standard preparations with each use.

3. Transferrin Tracer

Reconstitute the lyophilized transferrin tracer in 600 µL of Tracer Diluent to a 10x concentration. Dilute to 1x with Tracer Diluent within 30 minutes of running the assay. Store the unused reconstituted 10x concentrate at -20°C. It is stable for 3 freeze-thaw cycles.

4. Transferrin Antibody

Reconstitute the lyophilized transferrin antibody in 600 μ L Antibody Diluent to a 10x concentration. Dilute to 1x with Antibody Diluent within 30 minutes of running the assay. Store the unused reconstituted 10x concentrate at -20°C . It is stable for 3 freeze-thaw cycles.

ASSAY PROCEDURE

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove unneeded wells and return them, with the desiccant, to the plate bag and seal. Store the unused wells at 4°C .

1. Add 100 μ L of the assay buffer into the Bo (0 ng/ml standard) wells and 150 μ L of the same assay buffer into the NSB wells.
2. Add 100 μ L of Standards #1 through #6 into the appropriate wells.
3. Add 100 μ L of the Samples into the appropriate wells.
4. Add 50 μ L of the transferrin tracer to all wells except for the blank.
5. Add 50 μ L of the transferrin antibody to all wells except for the NSB and blank.
6. Seal the plate and incubate at room temperature (RT) on a plate shaker for 1 hour at $\sim 500\text{rpm}^*$. **See note.**
7. Empty the contents of the wells and wash by adding a full well volume (~ 400 μ L) of wash solution to every well. Repeat the wash 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
8. Add 200 μ L of the SA-HRP conjugate into each well except the blank.
9. Seal the plate and incubate at RT on a plate shaker for 30 minutes at $\sim 500\text{rpm}^*$.
10. Wash as above (Step 7).
11. Add 200 μ L of the TMB substrate solution into each well. Incubate for 30 minutes at RT on a plate shaker at $\sim 500\text{rpm}^*$.
12. Add 50 μ L of the stop solution into each well.

13. After zeroing the plate reader against the blank, read optical density at 450nm. If the plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the blank from all readings.

*** The plate shaker speed was based on a BellCo Mini Orbital Shaker (mod no. 7744-08096). The actual speed of the plate shaker should be such that the liquid in the plate wells mixes thoroughly, but does not splash out of the well.**

CALCULATION OF RESULTS

The concentration of transferrin can be calculated as follows:

1. Calculate the average net OD for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

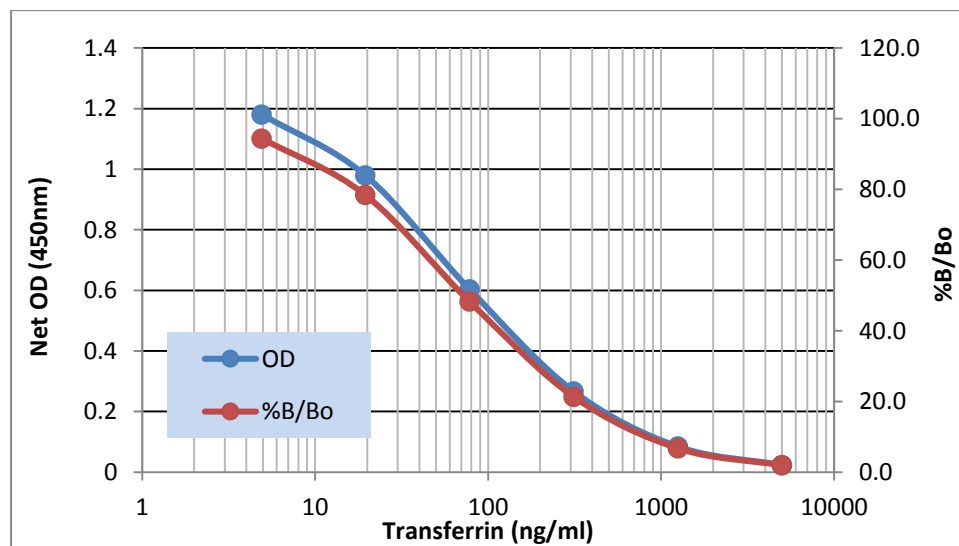
$$\text{Average Net OD} = \text{Average OD} - \text{Average NSB OD}$$

2. Using data analysis software, plot the Average Net OD for each standard versus transferrin concentration in each standard. We recommend that the data be handled by a software package utilizing a 4 parameter logistic (4PL) curve fitting program.

TYPICAL RESULTS

The results shown below are for illustration only and should not be used to calculate results.

Sample	Optical Density (450nm)	Binding (%B/Bo)	Transferrin (ng/mL)
NSB	0.029		n/a
Bo	1.25		0
S1	0.024	1.97	5000
S2	0.085	6.8	1250
S3	0.267	21.37	312.5
S4	0.604	48.35	78.1
S5	0.980	78.46	19.5
S6	1.181	94.45	4.9



PERFORMANCE CHARACTERISTICS

Specificity

The specificity of the assay was determined by running serial dilutions of the analytes, including the cross-reactant, in the assay, fitting the resulting dose response curve(s) to a 4PL curve-fit and determining the ED₅₀. The ED₅₀ of the standard curve was then divided by the determined ED₅₀ of the cross-reactant and multiplied by 100.

Analyte	Cross Reactivity
Transferrin	100%
Lactoferrin	0.014%

Sensitivity

The sensitivity or limit of detection of the assay is 4.6ng/mL, determined by interpolation at 2 standard deviations away from the net OD of 16 zero standard replicates. Data was used from 9 standard curves.

Intra-assay precision was determined by assaying 20 replicates of three matrix controls containing transferrin in a single assay.

Intra-assay precision	
ng/ml	%CV
358.7	4.8
65.5	4.9
12.2	9.4

Inter-assay precision was determined by measuring matrix controls of varying transferrin concentrations in multiple assays over several days.

Inter-assay precision	
ng/mL	%CV
389.7	8.5
71	14.3
11.9	19.6

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