TF (Human) ELISA Kit

Catalog Number KA0510

96 assays

Version: 07

Intended for research use only

Table of Contents

Introduction

Background

Transferrin is a plasma protein that transports iron through the blood to the liver, spleen and bone marrow. Low transferrin level in plasma could associate with anemia (1) and chronic liver disease (2). On the other hand, high plasma transferrin level could indicate iron deficiency anemia (3).

Principle of the Assay

The TF (Human) ELISA Kit is designed for detection of human transferrin in urine, milk, saliva, CSF, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures transferrin in less than 4 hours. A polyclonal antibody specific for transferrin has been pre-coated onto a 96-well microplate with removable strips. Transferrin in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for transferrin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

General Information

Materials Supplied

List of component

Component	Amount
Human Transferrin Microplate: A microplate coated with a polyclonal antibody against human Transferrin.	96 (8x12) wells
Sealing Tapes: Pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.	3 slides
Human Transferrin Standard: Human Transferrin in a buffered protein base (lyophilized).	400ng
Biotinylated Transferrin Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against transferring.	140 µl
MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base.	30 ml
Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant.	30 ml x 2
Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate.	80 µl
Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine.	8 ml
Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction.	12 ml

Storage Instruction

- ✓ Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- ✓ Store SP Conjugate and Biotinylated Antibody at -20°C.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- ✓ Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- ✓ Diluent (1x) may be stored for up to 30 days at 2-8°C.
- ✓ Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm
- ✓ Pipettes (1-20 µl, 20-200 µl, 200-1000 µl and multiple channel)
- ✓ Deionized or distilled reagent grade water

Precautions for Use

- ✓ Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated-antibody, and SP conjugate) as instructed, prior to running the assay.
- ✓ Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.
- ✓ Spin down the SP conjugate vial and the biotinylated-antibody vial before opening and using contents.
- ✓ This kit is for research use only.
- ✓ The kit should not be used beyond the expiration date.
- ✓ The Stop Solution is an acidic solution.

Assay Protocol

Reagent Preparation

- ✓ Freshly dilute all reagents and bring all reagents to room temperature before use.
- ✓ MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- ✓ Standard Curve: Reconstitute the 400 ng of HumanTransferrin Standard with 4 ml of MIX Diluent to generate a stock solution of 100 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (100 ng/ml) 1:2 with MIX Diluent 50, 25, 12.5, 6.25, 3.125, and 1.563 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[Transferrin] (ng/ml)
P1	Standard (100 ng/ml)	100.000
P2	1 part P1 + 1 part MIX Diluent	50.000
P3	1 part P2 + 1 part MIX Diluent	25.000
P4	1 part P3 + 1 part MIX Diluent	12.500
P5	1 part P4 + 1 part MIX Diluent	6.250
P6	1 part P5 + 1 part MIX Diluent	3.125
P7	1 part P6 + 1 part MIX Diluent	1.563
P8	MIX Diluent	0.000

- ✓ Biotinylated Transferrin Antibody (50x): Spin down the biotinylated antibody briefly and dilute the desired amount of the antibody 1:50 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- ✓ Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- ✓ SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Sample Preparation

- ✓ Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.
- ✓ Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes and assay. Urine dilution is suggested at 1:4 in MIX Diluent; however, the user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- ✓ Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. Saliva

dilution is suggested at 1:200 in MIX Diluent; however, the user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- ✓ Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. Milk dilution is suggested at 1:400 in MIX Diluent; however, the user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- ✓ CSF: Collect cerebrospinal fluid (CSF) using sample tube. Centrifuge samples at 3000 x g for 10 minutes. Dilute samples 1:2000 into MIX Diluent and assay. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

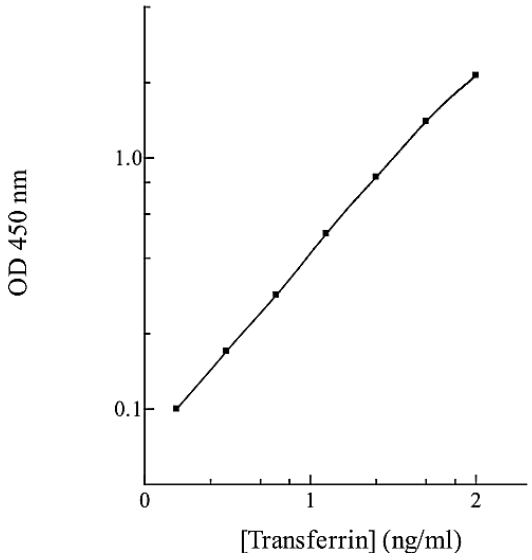
Assay Procedure

1. Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 50 µl of Human Transferrin Standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
4. Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents, hit it 4-5 times on absorbent material to completely remove the liquid. If using a machine wash six times with 300µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent material to completely remove the liquid.
5. Add 50 µl of Biotinylated Human Transferrin Antibody to each well and incubate for 1 hour.
6. Wash a microplate as described above.
7. Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash the microplate as described above.
9. Add 50 µl of Chromogen Substrate per well and incubate for about 12 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
10. Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
11. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

Calculation of Results

- ✓ Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- ✓ To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using four-parameter or log-log logistic curve fit.
- ✓ Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.



The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.

Performance Characteristics

- ✓ The minimum detectable dose of Transferrin is typically ~1.5 ng/ml.
- ✓ Intra-assay and inter-assay coefficients of variation were 4.8% and 7.1% respectively.

✓ Linearity

Average Percentage of Expected Value	
Sample Dilution	Urine
1:2	91%
1:4	98%
1:8	104%

Average Percentage of Expected Value		
Sample Dilution	Saliva	Milk
1:100	83%	N/A
1:200	97%	92%
1:400	104%	99%
1:800	N/A	105%

✓ Recovery

Standard Added Value	3.13 – 50 ng/ml
Recovery %	86-111%
Average Recovery %	96.5 %

✓ Cross-Reactivity

Species	**% Cross Reactivity**
Canine	None
Bovine	None
Monkey	< 5%
Mouse	None
Rat	None
Swine	None
Rabbit	None

10% FBS in culture media will not affect the assay.

Resources

References

1. Averbukh Z et. al. (2004) J Nephrol. 17(1): 101-6
2. Valberg LS et. al. (1978) Can Med Assoc J. 119(3): 229-36
3. Akinkugbe FM et. al. (1999) Afr J Med Med Sci. 28(1-2):25-9

Plate Layout

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