



## AssayMax Human Transferrin ELISA Kit (Urine, Milk, Saliva and Cell Culture Samples)

Catalog No. ET3105-1

### Introduction

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).

### Principal of the Assay

The AssayMax Human Transferrin ELISA kit is designed for detection of human transferrin in urine, milk, saliva, and cell culture supernatants. 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 microplate. 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.

### Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

### Reagents

- **Human Transferrin Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Transferrin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human Transferrin Standard:** Human Transferrin in a buffered protein base (1 µg, lyophilized).
- **Biotinylated Transferrin Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against transferrin (80 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).

- **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 Condition

- Store kit at 2-8<sup>0</sup>C or -20<sup>0</sup>C upon arrival up to the expiration date.
- Opened MIX Diluent may be stored for up to 1 month at 2-8<sup>0</sup>C. Store reconstituted reagents at -20<sup>0</sup>C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

## Other Supplies Required

- 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.

## Sample Collection, Preparation and Storage

- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20<sup>0</sup>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<sup>0</sup>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<sup>0</sup>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<sup>0</sup>C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

## Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8<sup>0</sup>C.
- **Standard Curve:** Reconstitute the 1 µg of Transferrin Standard with 2.5 ml of MIX Diluent to generate a stock solution of 400 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 (400 ng/ml) 1:4 with MIX Diluent to produce 100 ng/ml. Create the standard curve by serially diluting 1:2 with MIX Diluent to produce 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.

Standard Point	Dilution	[Transferrin] (ng/ml)
P1	Standard (400 ng/ml) + 3 parts MIX Diluent	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 (100x):** Spin down the biotinylated antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** 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.

## Assay Procedure

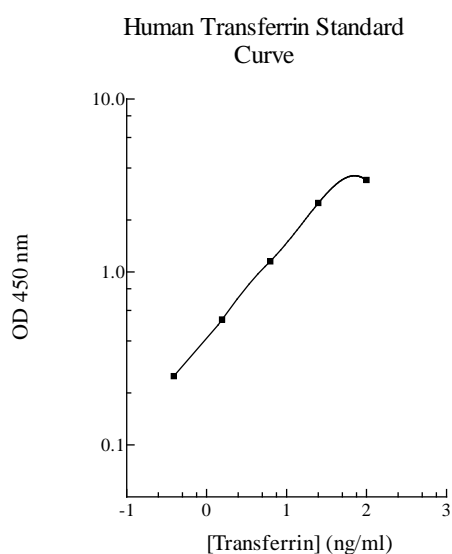
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
- 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 paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
- Add 50 µl of Biotinylated Transferrin Antibody to each well and incubate for one hour.
- Wash a microplate as described above.
- 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.
- Wash a microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 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.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- 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

- 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 log-log or semi-log curve fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the urine value by the dilution factor.

## Standard Curve

- 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 0.2 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.5% and 7.1% respectively.

## Linearity

Sample Dilution	Average Percentage of Expected Value
	Urine
1:2	101%
1:4	100%
1:8	98%

## Recovery

Standard Added Value	2 – 50 ng/ml
Recovery %	84-112 %
Average Recovery %	96 %

## Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Bovine	None
Monkey	< 20
Mouse	None
Rat	None
Swine	None

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

## 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

Version 3.5R1

## Related Products

- ET2105-1 AssayMax Human Transferrin ELISA Kit (Plasma and Serum Samples)