



AssayMax™

Rat Transferrin ELISA Kit

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For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Assay Summary

Step 1. Add 50 μ l of Standard or Sample per well.
Incubate 2 hours.

Step 2. Wash, then add 50 μ l of Biotinylated Antibody per well.
Incubate 1 hour.

Step 3. Wash, then add 50 μ l of SP Conjugate per well.
Incubate 30 minutes.

Step 4. Wash, then add 50 μ l of Chromogen Substrate per well.
Incubate 10 minutes.

Step 5. Add 50 μ l of Stop Solution per well.
Read at 450 nm immediately.

Symbol Key



Consult instructions for use.

Rat Transferrin ELISA Kit

Catalog No. ERT3105-1

Sample insert for reference use only

Introduction

Transferrin is a plasma protein that transports iron through the blood to the liver, spleen, and bone marrow (1).

Principle of the Assay

The AssayMax Rat Transferrin ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of rat transferrin in **urine and cell culture samples**. This assay employs a quantitative **sandwich enzyme immunoassay** technique that measures rat transferrin in less than 4 hours. A polyclonal antibody specific for rat transferrin has been pre-coated onto a 96-well microplate with removable strips. Rat transferrin in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for rat transferrin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is 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 product is for **Research Use Only** and is Not For Use In Diagnostic Procedures.
- Prepare all reagents (working diluent buffer, wash buffer, standard, 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 insert. 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.
- The Stop Solution is an acidic solution.
- The kit should not be used beyond the expiration date.

Reagents

- **Rat Transferrin Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against rat transferrin.

- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Rat Transferrin Standard:** Rat transferrin in a buffered protein base (30 ng, lyophilized).
- **Biotinylated Rat Transferrin Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against rat transferrin (80 μ l).
- **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, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 μ l).
- **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

- 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.

Other Supplies Required

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

Sample Collection, Preparation, and Storage

- **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. Urine is recommended for use at 1:100 into MIX Diluent or within the range of 20x-200x, and assay. Depending on application needs, user should determine proper dilutions. The undiluted samples can be stored at -20°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.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 30 ng of Rat Transferrin Standard with 1.5 ml of MIX Diluent to generate a 20 ng/ml standard stock solution. 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 stock solution (20 ng/ml) 1:2 with MIX Diluent to produce 10, 5, 2.5, 1.25, 0.625, and 0.313 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 the next 30 days.

Standard Point	Dilution	[Rat Transferrin] (ng/ml)
P1	1 part Standard (20 ng/ml)	20.00
P2	1 part P1 + 1 part MIX Diluent	10.00
P3	1 part P2 + 1 part MIX Diluent	5.000
P4	1 part P3 + 1 part MIX Diluent	2.500
P5	1 part P4 + 1 part MIX Diluent	1.250
P6	1 part P5 + 1 part MIX Diluent	0.625
P7	1 part P6 + 1 part MIX Diluent	0.313
P8	MIX Diluent	0.000

- **Biotinylated Rat 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):** 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.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 μ l of Rat Transferrin Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 μ l of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 μ l of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 μ l of Biotinylated Rat Transferrin Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 μ l of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 μ l of Chromogen Substrate per well and incubate for 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 μ l 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

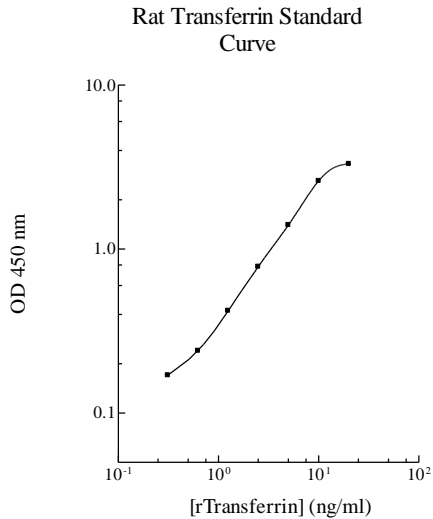
- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm

absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.

- Determine the unknown sample concentration from the Standard Curve and multiply the 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 rat transferrin is typically ~ 0.3 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.7% and 7.0% respectively.

Linearity

Average Percentage of Expected Value (%)	
Sample Dilution	Urine
1:50	103%
1:100	97%
1:200	107%

Recovery

Standard Added Value	1 – 10 ng/ml
Recovery %	84 – 112%
Average Recovery %	96.5%

Cross-Reactivity

Species	Cross Reactivity (%)
Canine	None
Bovine	None
Monkey	None
Mouse	20%
Rat	100%
Swine	None
Rabbit	None
Human	None

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

Reference

(1) Averbukh Z *et. al.* (2004) *J Nephrol.* 17(1): 101-6

Version 1.1R

Related Products

- ERT2105-1 AssayMax Rat Transferrin ELISA Kit (Plasma and Serum samples)
- ET2105-1 AssayMax Human Transferrin ELISA Kit (Plasma and Serum samples)
- ET3105-1 AssayMax Human Transferrin ELISA Kit (Urine, Milk, Saliva, and Cell Culture samples)
- EMT2105-1 AssayMax Mouse Transferrin ELISA Kit (Plasma, Serum, and Cell Culture samples)