# Rat Transferrin
# ELISA Kit

**Vertrieb:**
**L O X O GmbH** Immunbiologie Biochemie, Produkte und Systeme
Postfach 11 30 69215 Dossenheim
**Telefon** +49 (0) 62 21 - 86 80 23 **FAX** +49 (0) 62 21 - 86 80 255
**E-Mail:** info@loxo.de **Internet:** www.loxo.de

Assaypro LLC
30 Triad South Drive
St. Charles, MO 63304
T (636) 447-9175
F (636) 447-9475
www.assaypro.com

**Hinweis/Note:**
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

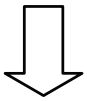
The datasheet is only a first information.
The relevant datasheet is included with the product.

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

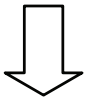
Thank you for choosing Assaypro.

## Assay Summary

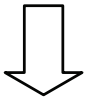
Add 25 µl of Standard/ Sample
and 25 µl of Biotinylated Protein per well.
Incubate 2 hours.



Wash, then add
50 µl of SP Conjugate per well.
Incubate 30 minutes.



Wash, then add
50 µl of Chromogen Substrate per well.
Incubate 12 minutes.



Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

# Assay Template

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | |
| 2 | | | | | | | | |
| 3 | | | | | | | | |
| 4 | | | | | | | | |
| 5 | | | | | | | | |
| 6 | | | | | | | | |
| 7 | | | | | | | | |
| 8 | | | | | | | | |
| 9 | | | | | | | | |
| 10 | | | | | | | | |
| 11 | | | | | | | | |
| 12 | | | | | | | | |

# AssayMax Rat Transferrin ELISA Kit

Catalog No. ERT2105-1
Sample Insert/Reference Only

## Introduction

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

## Principle of the Assay

The AssayMax Rat Transferrin ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of rat transferrin in plasma and serum samples. This assay employs a quantitative competitive enzyme immunoassay technique that measures rat transferrin in less than 3 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 competed with a biotinylated rat transferrin sandwiched by the immobilized antibody and 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

- **Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated protein, 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 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.

## 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 (20 μg, lyophilized).
- **Biotinylated Rat Transferrin:** 1 vial, lyophilized.
- **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

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate 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 and Biotinylated Protein 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 μl, 20-200 μl, 200-1000 μl, and multiple channel).
- Deionized or distilled reagent grade water.

## Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:3000 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes, and remove serum. Dilute samples 1:3000 into MIX Diluent and assay. 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 20 μg of Rat Transferrin Standard with 2 ml of MIX Diluent to generate a 10 μg/ml standard 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 solution (10 μg/ml) 1:2 with MIX Diluent to produce 5, 2.5, 1.25, 0.625, 0.313, and 0.156 μg/ml solutions. MIX Diluent serves as the zero standard (0 μg/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

| Standard Point | Dilution | [Rat Transferrin] (μg/ml) |
|---|---|---|
| P1 | Standard (10 μg/ml) | 10.00 |
| P2 | 1 part P1 + 1 part MIX Diluent | 5.000 |
| P3 | 1 part P2 + 1 part MIX Diluent | 2.500 |
| P4 | 1 part P3 + 1 part MIX Diluent | 1.250 |
| P5 | 1 part P4 + 1 part MIX Diluent | 0.625 |
| P6 | 1 part P5 + 1 part MIX Diluent | 0.313 |
| P7 | 1 part P6 + 1 part MIX Diluent | 0.156 |
| P8 | MIX Diluent | 0.000 |

- **Biotinylated Rat Transferrin (4x):** Reconstitute Biotinylated Rat Transferrin with 4 ml MIX Diluent to produce a 4-fold stock solution. Allow to sit for 10 minutes with gentle agitation prior to making dilutions. The stock solution should be further diluted 1:4 with MIX Diluent. Any remaining solution should be frozen at -20°C and used within 30 days.
- **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 25 μl of Rat Transferrin Standard or sample per well, and immediately add 25 μl of Biotinylated Rat Transferrin to each well (on top of the standard or sample) and mix gently. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 μl 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 μl 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 μl of Streptavidin-Peroxidase Conjugate to each 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 μl of Chromogen Substrate per well and incubate for 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.
- 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 low concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

## Data Analysis

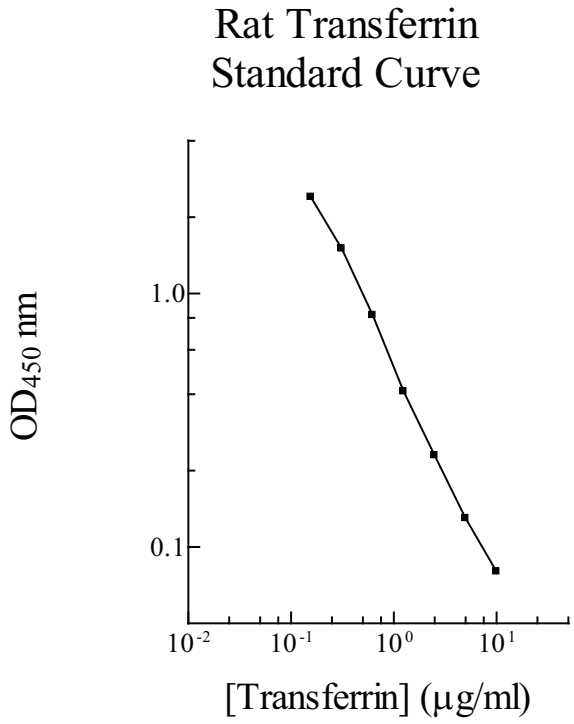
- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm

absorbance on the y-axis. The best-fit line can be determined by regression analysis using 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.

## 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 ~ 0.1 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.8% and 7.1% respectively.

## Linearity

| | Average Percentage of Expected Value | |
|---|---|---|
| **Sample Dilution** | **Plasma** | **Serum** |
| **1:1500** | 105% | 104% |
| **1:3000** | 98% | 97% |
| **1:6000** | 89% | 90% |

## Recovery

| Standard Added Value | 0.3 – 5.0 µg/ml |
|---|---|
| Recovery % | 82 – 117% |
| Average Recovery % | 96% |

## 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 1.0R2

## Related Products

- ERT3105-1 AssayMax Rat Transferrin ELISA Kit (Urine and Cell Culture samples)
- ET3105-1 AssayMax Human Transferrin ELISA Kit (Urine, Saliva, Milk, and Cell Culture samples)
- ET2105-1 AssayMax Human Transferrin ELISA Kit (Plasma and Serum samples)
- EMT2105-1 AssayMax Mouse Transferrin ELISA Kit (Plasma, Serum, and Cell Culture samples)