## PERFORMANCE CHARACTERISTICS (continued)

**Sample Recovery**

High and low concentrations of purified mouse transferrin were spiked into each of 3 serum samples. Observed assay values compared to expected values ranged from 72 to 116%, indicating accurate quantification of transferrin in mouse serum.

| Sample | Expected ng/ml | Observed ng/ml | Observed/ Expected |
|---|---|---|---|
| High Transferrin Spike | | 39.9 | |
| + Mouse D, 50.4 ng/ml | 90.3 | 92.4 | **102** |
| + Mouse E, 119 ng/ml | 159 | 159 | **100** |
| + Mouse F, 53.2 ng/ml | 93.2 | 100.6 | **108** |
| Low Transferrin Spike | | 8.95 | |
| + Mouse D, 50.4 ng/ml | 59.4 | 54.8 | **92** |
| + Mouse E, 119 ng/ml | 128 | 118 | **92** |
| + Mouse F, 53.2 ng/ml | 62.2 | 58.8 | **94** |

## Related Items

| Catalog# | ProdDescription |
|---|---|
| 6310 | Mouse IgA ELISA Kit, 96 tests, Quantitative |
| 6320 | Mouse IgG ELISA Kit, 96 tests, Quantitative |
| 6330 | Mouse IgG1 ELISA Kit, 96 tests, Quantitative |
| 6340 | Mouse IgG2a ELISA Kit, 96 tests, Quantitative |
| 6350 | Mouse IgG2b ELISA Kit, 96 tests, Quantitative |
| 6360 | Mouse IgG3 ELISA Kit, 96 tests, Quantitative |
| 6370 | Mouse IgE ELISA Kit, 96 tests, Quantitative |
| 6380 | Mouse IgM ELISA Kit, 96 tests, Quantitative |
| 6380-RS | Mouse IgM Reference Serum for ELISA (~500 ng/ml) |
| 6390 | Mouse Transferrin (Tf) ELISA Kit, 96 tests, Quantitative |
| 6390-20 | Rat Transferrin (Tf) ELISA Kit, 96 tests, Quantitative |
| 6390-30 | Rabbit Transferrin (Tf) ELISA Kit, 96 tests, Quantitative |
| 6390-40 | Chicken Ovotransferrin (Conalbumin) ELISA Kit, 96 tests, Quantitative |
| 6400 | Rat Albumin ELISA Kit, 96 tests, Quantitative |
| 6410 | Rat serum amyloid P (SAP) ELISA Kit, 96 tests, Quantitative |
| 6410-10 | Rat IgA ELISA kit 96 tests, Quantitative |
| 6420 | Rat IgG ELISA Kit, 96 tests, Quantitative |
| 6420-RDT-25 | TruStrip RDT Rat IgG Rapid Test cards,25/pk |
| 6430 | Rat IgG1 ELISA Kit, 96 tests, Quantitative |
| 6430 | cc# change to 6430-10; Rat interleulin-6 (rIL-6) ELISA Kit, 96 tests, Quantitative |
| 6430-10 | Rat interleulin-6 (rIL-6) ELISA Kit, 96 tests, Quantitative |
| 6430-20 | Mouse interleulin-6 (rIL-6) ELISA Kit, 96 tests, Quantitative |
| 6430-30 | Human interleulin-6 (hIL-6) ELISA Kit, 96 tests, Quantitative |
| 6440 | Rat IgG2a ELISA Kit, 96 tests, Quantitative |
| 6450 | Rat IgG2b ELISA Kit, 96 tests, Quantitative |
| 6470 | Rat IgE ELISA Kit, 96 tests, Quantitative |
| 6480 | Rat IgM ELISA Kit, 96 tests, Quantitative |
| 6490 | Rat Alpha-1 Glycoprotein (A1-AGP) ELISA kit 96 tests, Quantitative |



*Instruction Manual No. M-6390*

# Mouse Transferrin ELISA Kit

**Cat. No. 6390, 96 tests**

**For Quantitative Determination of Mouse Transferrin in Serum, Plasma or in other biological Fluids**

*For research use only (RUO), not for diagnosis, cure or prevention of the disease.*

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## INTENDED USE

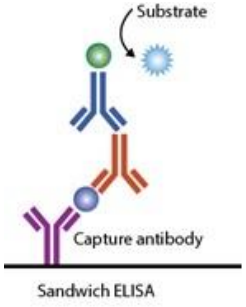
The Mouse Transferrin ELISA Kit is an in vitro immunoassay for the quantification of transferrin circulating in serum or in other appropriately qualified samples from tissue fluids (e.g., saliva, mucosa), or in cultures of mouse cells. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

## RESEARCH USE OF THE TEST

Elemental iron is required for a variety of normal cellular functions and vital for proper growth and development. However, natural iron is quite insoluble and excess iron is harmful, since it can catalyze the formation of potentially damaging reactive oxygen compounds. The mouse has a very limited capacity to excrete iron and cells have, therefore, developed mechanisms to improve solubility of iron and to control intracellular iron levels at the point of absorption in the intestine and other tissue. The major pool of body iron (~85%; 40-50 mg/kg) is found in circulating hemoglobin and muscle myoglobin. Several proteins including transferrin, transferrin receptors (TfRs), ferritin and iron regulatory proteins (IRPs) play a key role in iron metabolism.

Transferrin is a serum glycoprotein of ~80 kDa, synthesized in the liver, and is the primary protein of inter-organ transport of non-heme iron. It can bind two iron atoms and is normally about 30% iron-saturated to prevent accumulation of toxic iron. Transferrin (Tf) binds to TfRs and taken up by endocytosis. Iron is released within acidic endosomes into the cytoplasm, apparently through the action of DMT1. The apoTf-TfR complex is returned to the cell surface, where apo-Tf dissociates from TfR at the extra-cellular pH.

## PRINCIPLE OF THE TEST




The Mouse Transferrin ELISA kit is based on the binding of mouse transferrin in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to horseradish peroxidase (HRP) enzyme. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of transferrin present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of transferrin in samples and control is calculated from a curve of standards containing known concentrations of transferrin.

## STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8$^{o}$C until the expiration date printed on the label. Stabilities of the working solutions are indicated under Reagent Preparation.



## PERFORMANCE CHARACTERISTICS

The antibodies used in this kit have been shown by immunoelectrophoresis and ELISA to react specifically with transferrin, and have essentially no reactivity with any other mouse serum proteins.

Serum from the following species showed no significant reactivity at 1:100 dilution: human, rat, hamster, guinea pig, bovine, pig, horse, sheep, goat, dog, cat, rabbit or chicken; also 10% neonatal bovine serum.

**Normal Range**
Assay of transferrin in stored sera from twenty (20) individual Swiss mice ranged from 0.55 to 10.2 mg/ml (median = 5mg/ml). Each laboratory should determine expected values of its own testing population.

**Precision**
Samples containing low, medium and high concentrations of transferrin were assayed multiple times in the same assay (n=10) to provide within-assay precision, and as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficient of variations were calculated for the concentrations using a point-to-point curve-fitting program.

Transferrin concentrations were measured with very good within-assay (2.7 to 4.9 %CV) and between-assay (6.0 to 8.4 %CV) reproducibility.

| Sample | Transferrin ng/ml | Intra-assay %CV | Inter-assay %CV |
|---|---|---|---|
| Low Sample | 28 | **2.7** | **7.5** |
| Medium Sample | 73 | **4.9** | **6.0** |
| High Sample | 113 | **3.7** | **8.4** |

**Linearity of Dilution**
Three (3) individual and two (2) pooled stored sera were diluted to 2 levels for testing, and concordance of the assay values were compared. The mean recovery ranged from 80 to 100%, demonstrating linear dilution and equivalent quantification across the standard range.

| Sample | Dilution | Assay Value ng/ml | Serum Value mg/ml | Concordance |
|---|---|---|---|---|
| Mouse D | 1:25k<br>1:200k | 178<br>22 | 4.46<br>4.47 | **100 %** |
| Mouse E | 1:50k<br>1:400k | 164<br>30.5 | 8.19<br>12.2 | **80 %** |
| Mouse F | 1:25k<br>1:200k | 174<br>24.4 | 4.34<br>4.89 | **94 %** |
| Mouse Pool BM | 1:20k<br>1:80k | 192<br>71 | 3.83<br>5.66 | **81 %** |
| Mouse Pool BL | 1:20k<br>1:160k | 141<br>16 | 2.82<br>2.54 | **95 %** |

## CALCULATION OF RESULTS

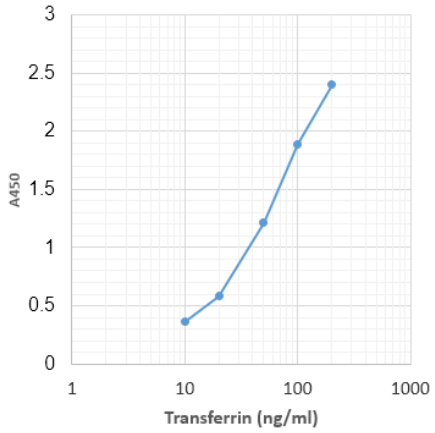
1. The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, Trf concentrations may be determined as follows:
2. Calculate the mean OD of duplicate samples.
3. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of Trf (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
4. The Trf concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
5. Multiply the values obtained for the samples by the dilution factor of each sample.
6. Samples producing signals higher than the 200 ng/ml standard should be further diluted and re-assayed.

## TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

| Wells | Standards, Control & Samples | A450 nm | **Trf** ng/ml |
|---|---|---|---|
| A1, A2 | **Negative Diluent Control** | 0.08 | 0 |
| B1, B2 | 10 ng/ml **Standard** | 0.36 | 10 |
| C1, C2 | 20 ng/ml **Standard** | 0.58 | 20 |
| D1, D2 | 50 ng/ml **Standard** | 1.21 | 50 |
| E1, E2 | 100 ng/ml **Standard** | 1.89 | 100 |
| F1, F2 | 200 ng/ml **Standard** | 2.40 | 200 |
| G1, G2 | **Positive Serum Control** [Value: 22 - 42 ng/ml] | 0.82 | 28 |
| H1, H2 | **Sample** [Diluted 1:100k] | 1.30 | 55 |
| | Calculated: 100k-fold dilution x 55 ng/ml = **5.50** mg/ml in serum | | |

A typical assay Standard Curve (do not use for calculating sample values)




Bill/6390-Mouse-Tf-ELISA-Graph



## KIT CONTENTS

**To Be Reconstituted:** Store as indicated.

| Component | Instructions for Use |
|---|---|
| **Sample Diluent Concentrate (20x)** Cat. No. SD-20T, 10ml | Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8ºC until the kit lot expires or is used up. |
| **Wash Solution Concentrate (100x)** Cat. No. WB-100, 10ml | Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at RT until kit is used entirely. |
| **Anti-Mouse Trf - HRP Conjugate Concentrate (100x)** Part No. 6394, 0.15ml | Peroxidase conjugated anti-mouse Trf in buffer with protein, detergents and ProClin 300 as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8ºC storage. |

**Ready For Use:** Store as indicated on labels.

| Component | Part No. | Amt | Contents |
|---|---|---|---|
| **Anti-Mouse Trf Microwell Strip Plate** | 6391 | 8-well strips (12) | Coated with purified anti-mouse transferrin antibodies. |
| **Mouse Transferrin Standards** | | | |
| 10 ng/ml<br>20 ng/ml<br>50 ng/ml<br>100 ng/ml<br>200 ng/ml | 6393B<br>6393C<br>6393D<br>6393E<br>6393F | 0.65 ml<br>0.65 ml<br>0.65 ml<br>0.65 ml<br>0.65 ml | Five (5) vials, each containing calibrated, purified mouse transferrin; diluted in buffer with protein, detergents and ProClin 300 as stabilizers. |
| **Positive Control** [Transferrin] range on label | 6392 | 0.65 ml | Mouse serum transferring with stated concentration range; diluted in buffer with protein, detergents and ProClin 300 as stabilizers. |
| **TMB Substrate** | 80091 | 12 ml | Chromogenic substrate for HRP containing TMB and peroxide. |
| **Stop Solution** | 80101 | 12 ml | 1% sulfuric acid. |

**Materials Required But Not Provided:**

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipetter is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-mouse Trf-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

## SPECIMEN COLLECTION AND HANDLING

Culture medium, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, including tissue culture media, clarify the sample by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately, stored refrigerated for up to a few weeks, or frozen for long-term storage. Avoid freeze-thaw cycles.

## PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Controls, Sample Diluent, and Anti-mouse Trf-HRP contain Proclin 300 (0.05%, v/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the web site for Prolcin-300 (0.1% v/v in standards, and assay buffers).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

## QUALITY CONTROL

**Reagents** Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

**Sample Controls** A Positive Serum Control is provided with the kit, assigned with an Trf concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Negative Diluent Control should also be run.



## ASSAY PROCEDURE

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

DILUTE Serum Samples in Working Sample Diluent. Dilutions of about 20-50k-fold are appropriate for most normal mouse sera. For accuracy, two dilution steps are recommended, as follows:

1) 10ul serum + 990ul diluent = [1:100],
2) 5ul [1:100] + 995ul diluent = **[1:20k].**

DO NOT diluted the Standards or Positive Control.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

**1. Set-up**

- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for ~5 minutes before sample addition.
- Aspirate or dump the liquid and pat dry on a paper towel.

**2. 1st Incubation [100ul - 60min; 4 washes]**

- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer is recommended. Improper washes may lead to falsely elevated signals and poor reproducibility.

**3. 2nd Incubation [100ul - 30min; 5 washes]**

- Add 100ul of diluted Anti-mouse Trf-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

**4. Substrate Incubation [100ul - 15min]**

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.
  Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, assuring the top standard does not surpass 2 OD.

**5. Stop Step [Stop: 100ul]**

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

**6. Absorbance Reading**

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.