RAT TRANSFERRIN ELISA
Life Diagnostics, Inc., Catalog Number: TF-2

INTRODUCTION

Transferrin is a glycosylated serum protein with a molecular weight of 80,000 that serves as an iron carrier in blood. In rats it is a negative acute phase reactant, the serum levels of which decrease by 25 - 40% within 18-24 hours of induction of the acute phase response.[1,2]

PRINCIPLE OF THE ASSAY

The assay uses affinity purified anti-transferrin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-transferrin antibodies for detection. Standards and diluted samples are incubated in the microtiter wells for 45 minutes. The wells are subsequently washed. HRP conjugate is added and incubated for 45 minutes. This results in transferrin molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate and TMB is added and incubated for 20 minutes. If transferrin is present a blue color develops. Color development is stopped by the addition of Stop solution, changing the color to yellow, and absorbance is measured at 450 nm. The concentration of transferrin is proportional to absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Transferrin antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 ml
- Transferrin stock (lyophilized)
- 20x Wash solution; TBS50-20, 50 ml
- Diluent; YD25-10, 25 ml
- TMB, TMB11-1, 11 ml
- Stop solution, SS11-1, 11 ml

Materials required but not provided:

- Pipettors and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm.
- Curve fitting software

STORAGE

Store the kit at 2-8°C. The microtiter plate should be kept in a sealed bag with desiccant. The kit will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

DILUENT PREPARATION

The diluent is provided as a 10x stock. Estimate the final volume of diluent required for your assay. Dilute one (1) volume of the 10x stock with nine (9) volumes of distilled or deionized water to give the required volume.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The rat transferrin stock is provided lyophilized. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved.
2. Label 6 polypropylene or glass tubes as 00, 50, 25, 12.5, 6.25, and 3.125 ng/ml.
3. Into the tube labeled 100 ng/ml, pipette the volume of diluent detailed on the stock vial label. Then add the indicated volume of stock and mix. This provides the 100 ng/ml standard.
4. Dispense 250 µl of diluent into each of the tubes labeled 100, 50, 25, 12.5, 6.25, and 3.125 ng/ml.
5. Prepare the 50 ng/ml standard by diluting and mixing 250 µl of the 100 ng/ml standard with 250 µl of diluent in the tube labeled 50 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

Unused reconstituted reference stock should be stored frozen at or below -20°C if future use is intended.

SAMPLE PREPARATION

Transferrin is present in rat serum at concentrations of approximately 5 mg/ml. To obtain values within range of the standard curve we suggest that samples be diluted 200,000-fold using the following procedure for each sample to be tested.

1. Dispense 998 µl and 798 µl of 1x diluent into separate tubes.
2. Pipette and mix 2.0 µl of serum or plasma into the tube containing 998 µl of 1x diluent. This provides a 500-fold dilution.
3. Mix 2.0 µl of the 500-fold diluted sample with the 798 µl of 1x diluent in the second tube. This provides a 200,000-fold dilution.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 4°C for future use.
2. Dispense 100 µl of standards and samples into the wells (we recommend that standards and samples be run in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 45 minutes.

4. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 µl/well).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 µl of HRP-conjugate into each well.
7. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
8. Wash as detailed above.
9. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
10. Dispense 100 µl of TMB into each well.
11. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
12. After 20-minutes, stop the reaction by adding 100 µl of Stop solution to each well.
13. Gently mix. It is important to make sure that all the blue color changes to yellow.
14. Read absorbance at 450 nm with a plate reader within 5 minutes.

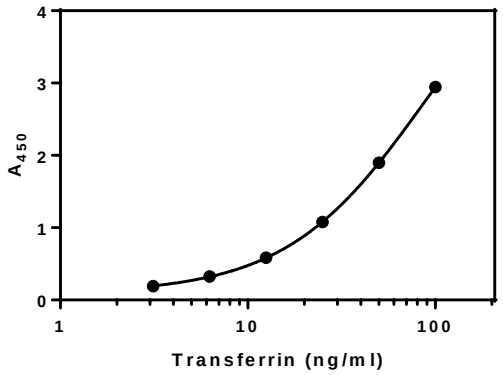
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus $\log_{10}$ of the concentration.
2. Fit the standard curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples from the standard curve (remember to derive the concentration from the antilog).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum or plasma sample.
4. If the A_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve is shown below. This curve is for illustration only and should not be used to calculate unknowns. A standard curve must be generated for each experiment.

Transferrin (ng/ml)	A_{450}
100	2.944
50	1.900
25	1.077
12.5	0.584
6.25	0.325
3.125	0.193



REFERENCES

1. Powanda MC, et al. Differential Effect of Clofibrate on Inflammation-Induced Alterations in Plasma Proteins in the Rat. Biochem J. 178:633-641 (1979)
2. Eberini I, et al. Proteins of rat serum IV. Time-course of acute-phase protein expression and its modulation by indomethacine. Electrophoresis. 20: 846-853 (1999)

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For technical assistance please email us at techsupport@lifediagnostics.com