RAT TRANSFERRIN RID KIT
Life Diagnostics, Inc. Cat. No. RID-1012/RID-1012X

Radial Immunodiffusion (RID) Assay for Measurement of Transferrin in Rat Serum or Plasma

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 (refs. 1 & 2). The high serum concentration of transferrin (~5 mg/ml in normal rat serum) complicates measurement by ELISA methods due to the extremely high sample dilutions (100,000 fold or greater) required to obtain values within range of the ELISA standard curve. In contrast, the transferrin RID assay offered by Life Diagnostics requires only a single ~40 fold sample dilution. Other advantages of RID compared to ELISA methodology include simplicity and the fact that no expensive equipment is required for performance of the assay.

PRINCIPLE OF THE TEST

Diluted rat serum or plasma samples (10 μl) are placed in wells of an agar plate containing antiserum against rat transferrin. As the sample diffuses radially from the well a precipitin reaction occurs between transferrin and anti-transferrin antibodies, resulting in the formation of a precipitin ring, the diameter of which is measured 24 hours after sample addition. The diameter of the precipitin ring is proportional to the concentration of transferrin in the sample. Transferrin concentration is determined by comparison to reference standards.

KIT CONTENTS

- RID plates with 10 test wells/plate (5 plates)
- Transferrin Standard, 0.3 mg/ml (Lyophilized) (1 vial)
- Sample Diluent, 25 ml (1 bottle)
- Magnifier with measuring reticle (included with kit RID-1012X)
- Resealable plastic bags (2)

MATERIALS REQUIRED BUT NOT SUPPLIED

- Micro centrifuge tubes for dilution of standards
- Pipettor / pipette tips
- 37°C Incubator (optional)
- Paper towels or filter paper
- Water
- Graph paper (or PC graphing software)

STORAGE OF TEST KIT

All components of the kit should be stored at 2-8°C prior to use.

TEST PROCEDURE

1. Reconstitute the 0.3 mg/ml rat transferrin standard as described on the vial label (unused reconstituted 0.3 mg/ml rat transferrin stock should be stored at or below –20°C).
2. Dispense 50 μl of Sample Diluent into each of three polypropylene microcentrifuge tubes and label them as 0.15, 0.075 and 0.038 mg/ml.
3. Add 50 μl of the 0.3 mg/ml rat transferrin standard to the tube labeled 0.15 mg/ml and mix. This constitutes the 0.15 mg/ml standard
4. Similarly prepare 0.075 and 0.038 mg/ml stocks by serial dilution.
5. Remove the plastic cover from an RID plate.
6. Pipette 10 μl of the 0.3, 0.15, 0.075 and 0.038 mg/m standards into separate wells on one of the agar plates **(a standard curve on one plate will suffice for all plates used in an experiment)**.
7. In an identical manner, pipette 10 μl of diluted rat serum samples into separate wells. Normal levels of transferrin in rat serum are approximately 5 mg/ml. We therefore suggest that serum samples be diluted 40-fold by mixing 2.5 μl of serum with 97.5 μl of diluent.
8. Allow the samples to **completely** adsorb into the agar by incubating the plate on the bench top at room temperature for 5-15 minutes.
9. Firmly secure the plate cover
10. Invert the plate (this minimizes the formation of condensation on the inner surface of the lid) and place it in a small resealable plastic bag together with a small piece of filter paper (or paper towel) moistened with water. Maintain the plate in a horizontal position.
11. Incubate at 37°C for 24 hours (alternatively, the plate may be incubated at room temperature for 48 hours).

EVALUATION OF RESULTS

1. Remove the plate from the plastic bag. Remove the lid if condensation has formed on its inner surface but be careful not to damage the agar.
2. Determine the optimum conditions for viewing the precipitin rings. The rings are best viewed from the bottom of the plate. We find that good contrast is obtained if the plate is viewed indirectly against a background of overhead fluorescent lighting.
3. Place the largest end of the magnifier against the bottom of the plate and measure the external diameter of each ring to the nearest 0.1 mm. Please note that the focal length of the magnifier can be adjusted.
4. Plot the squared value of the ring diameter (Y axis) versus the respective concentration of the standard (X axis). Draw a straight line through the points.
5. The concentration of transferrin in the test samples is derived from the intercept on the X axis corresponding to the squared value of the precipitin ring diameter of the test sample.

Life Diagnostics, Inc., P.O. Box 5205, West Chester, PA 19380
610-431-7707 – 610-431-7818 (Fax)
info@lifediagnostics.com – www.lifediagnostics.com

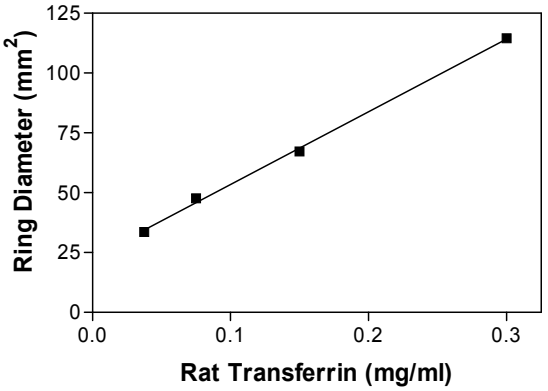
6. If using graphing software, a linear regression fit of the data should be performed.
7. If the test sample has been diluted, multiply the concentration determined from the standard curve by the dilution factor to obtain the actual concentration of transferrin.
8. If no precipitin ring appeared, or if the ring was too large in diameter, adjust the dilution factor and repeat the assay. The Sample Diluent should be used for dilution.

TYPICAL STANDARD CURVE

A typical standard curve is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

Transferrin (mg/ml)	Ring Diameter (mm)	mm^2
0.3	10.7	114.49
0.15	8.2	67.24
0.075	6.9	47.61
0.038	5.8	33.64

Typical Rat Transferrin Standard Curve



PRECAUTIONS

1. To ensure uniformity, the test sample should be thoroughly mixed prior to application to the well.
2. While adding the sample to the well, take care not to damage the agar gel.
3. Use separate pipette tips for each sample. If using a micro dispenser or syringe wash it thoroughly between samples.
4. Prior to use, remove any residual moisture in the test wells by allowing the uncovered plate to dry briefly at room temperature.
5. Repeated opening of the refrigerator door or fluctuations in temperature during storage might result in condensation of moisture on the gel surface that may cause inaccurate readings.

SHELF LIFE

1. Test kits are usable for at least six months from the date of manufacture when properly stored. DO NOT FREEZE THE RID PLATES.
2. Expiration date is recorded on the outside of the package.

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)