

# MOUSE TRANSFERRIN RID KIT

## Life Diagnostics, Inc. Cat. No. RID-1011/RID-1011X

### Radial Immunodiffusion (RID) Assay for Measurement of Transferrin in Mouse 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 contrast to rat and human transferrin which are negative acute phase reactants (their serum levels decrease during the acute phase response) mouse transferrin appears to be a positive acute phase reactant (ref. 1). The high serum concentration of transferrin (~5 mg/ml in normal mouse 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 mouse serum or plasma samples (10  $\mu$ l) are placed in wells of an agar plate containing antiserum against mouse 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 36-48 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, 200  $\mu$ g/ml (Lyophilized) (1 vial)
- Sample Diluent, 25 ml (1 bottle)
- Magnifier with measuring reticle (included with kit RID-1011X)
- 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 200  $\mu$ g/ml mouse transferrin standard as described on the vial label (unused reconstituted 200  $\mu$ g/ml mouse transferrin stock should be stored at or below -20°C).
2. Dispense 50  $\mu$ l of Sample Diluent into each of three polypropylene microcentrifuge tubes and label them as 100, 50 and 25  $\mu$ g/ml.
3. Add 50  $\mu$ l of the 200  $\mu$ g/ml mouse transferrin standard to the tube labeled 100  $\mu$ g/ml and mix. This constitutes the 100  $\mu$ g/ml standard
4. Similarly prepare 50 and 25  $\mu$ g/ml stocks by serial dilution.
5. Remove the plastic cover from an RID plate.
6. Pipette 10  $\mu$ l of the 200, 100, 50 and 25  $\mu$ g/ml 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  $\mu$ l of diluted mouse serum samples into separate wells. Normal levels of transferrin in mouse serum are approximately 5 mg/ml. We therefore suggest that serum samples be diluted 40-fold by mixing 2.5  $\mu$ l of serum with 97.5  $\mu$ l of diluent.
8. Allow the samples to adsorb into the agar by incubating the plate on the bench top at room temperature for 5-10 minutes.
9. Firmly secure the plate cover
10. Maintaining the plate in a horizontal position, place it in a small resealable plastic bag together with a small piece of filter paper (or paper towel) moistened with water and incubate at 37°C for 36-48 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.
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.

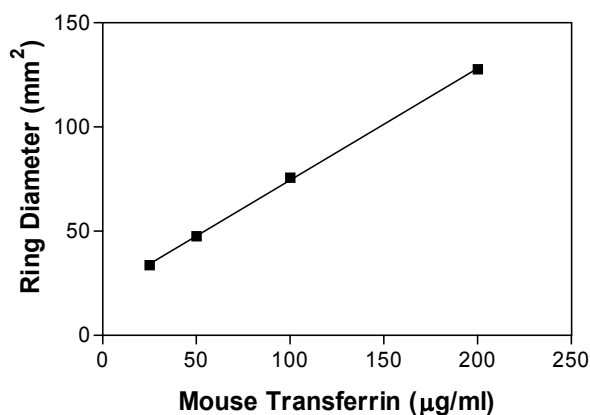
- If no precipitin ring appeared, or if the ring was too large in diameter, adjust the dilution factor and repeat the assay. The Sample Diluent should be used for dilution.

### 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 (µg/ml)	Ring Diameter (mm)	mm <sup>2</sup>
200	11.3	127.69
100	8.7	75.69
50	6.9	47.61
25	5.8	33.64

### Typical Mouse Transferrin Standard Curve



### REFERENCES

- Gordeuk VR, et.al., Interleukin 1 administration in mice produces hypoferrremia despite neutropenia. J. Clin. Invest. 82:1934-1938 (1988)

### PRECAUTIONS

- To ensure uniformity, the test sample should be thoroughly mixed prior to application to the well.
- While adding the sample to the well, take care not to damage the agar gel.
- Use separate pipette tips for each sample. If using a micro dispenser or syringe wash it thoroughly between samples.
- Prior to use, remove any residual moisture in the test wells by allowing the uncovered plate to dry briefly at room temperature.
- 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

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