

# RAT ALBUMIN RID KIT

## Life Diagnostics, Inc. Cat. No. RID-1002/RID-1002X

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

#### INTRODUCTION

Albumin is the most abundant protein present in serum. It is a negative acute phase reactant, the serum levels of which decrease by approximately 30% in response to disease, tissue injury or inflammation (1-3). The high serum concentration of albumin (~35 mg/ml in normal rat serum) complicates measurement by ELISA methods due to the extremely high sample dilutions (500,000 fold or greater) required to obtain values within range of the standard curve. In contrast, the albumin RID assay 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 (5  $\mu$ l) are placed in wells of an agar plate containing antiserum against rat albumin. As the sample diffuses radially from the well a precipitin reaction occurs between albumin and anti-albumin 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 albumin in the sample. Albumin concentration is determined by comparison to reference standards.

#### KIT CONTENTS

- RID plates with 10 test wells/plate (5 plates)
- Albumin Standard, 1.5 mg/ml (Lyophilized) (1 vial)
- Sample Diluent, 25 ml (1 bottle)
- Magnifier with measuring reticle (included with kit RID-1002X)
- 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 1.5 mg/ml rat albumin standard as described on the vial label (unused reconstituted 1.5 mg/ml rat albumin 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 0.75, 0.375 and 0.188 mg/ml.
3. Add 50  $\mu$ l of the 1.5 mg/ml rat albumin standard to the tube labeled 0.75 mg/ml and mix. This constitutes the 0.75 mg/ml standard
4. Similarly prepare 0.375 and 0.188 mg/ml stocks by serial dilution.
5. Remove the plastic cover from an RID plate.
6. Pipette 5  $\mu$ l of the 1.5, 0.75, 0.375 and 0.188 mg/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 5  $\mu$ l of diluted rat serum samples into separate wells. Normal levels of albumin in rat serum are approximately 35 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 **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 albumin 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.

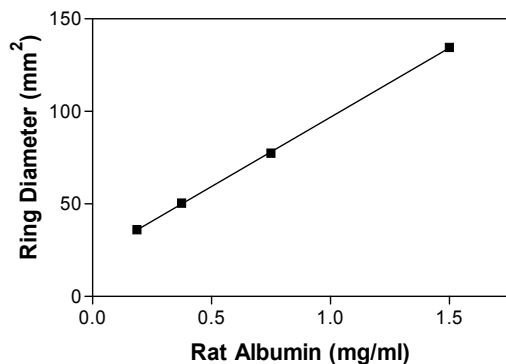
7. If the test sample has been diluted, multiply the concentration determined from the standard curve by the dilution factor to obtain the actual concentration of albumin.
8. If no precipitin ring appeared, or if the ring was too large in diameter, adjust the dilution factor and repeat the assay. The 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.

Albumin (mg/ml)	Ring Diameter (mm)	mm <sup>2</sup>
1.5	11.6	134.56
0.75	8.8	77.44
0.375	7.1	50.41
0.188	6.0	36

### Typical Rat Albumin 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. Aldred AR and Schreiber G. The negative acute phase proteins. pp 21-37. Acute Phase Proteins: Molecular Biology, Biochemistry and Clinical Applications. Eds. Mackiewicz A, Kushner I, and Baumann H. CRC Press (1993)
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)
3. Whalen R, Voss SH and Boyer TD. Decreased expression levels of rat liver glutathione S-transferase A2 and albumin during the acute phase response are mediated by HNF1 (hepatic nuclear factor 1) and IL6DEX-NP. Biochem J. 377:763-768 (2004)