

Rat Anti-SRBC IgG ELISA

Life Diagnostics, Inc., Catalog Number: SRBCG-2

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INTRODUCTION

Recent studies have demonstrated that suppression of anti-SRBC (sheep red blood cell) immunoglobulin levels by therapeutic agents serves as a useful indicator of immunosuppression.^{1,2} This test kit allows rapid and quantitative measurement of rat anti-SRBC IgG levels in serum or plasma samples.

PRINCIPLE OF THE ASSAY

The rat anti-SRBC IgG test kit is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses detergent solubilized SRBC ghosts² for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-rat IgG antibodies for detection. Test serum or plasma samples are diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed, and HRP conjugate is added and incubated for 45 minutes. Anti-SRBC IgG molecules are thus sandwiched between immobilized SRBC antigens and the detection antibody conjugate. The wells are then washed to remove unbound HRP-labeled antibodies. TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow. Optical density is measured spectrophotometrically at 450 nm. The concentration of anti-SRBC IgG in a test sample is proportional to the optical density and is derived by reference to a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- SRBC Coated 96-well Plate (provided as 12 strips of 8 wells)
- Enzyme Conjugate Reagent, 11 ml
- Reference Standard Stock^A (lyophilized)
- 20x Wash Solution: TBS50-20, 50 ml
- Diluent: YD30-1, 30 ml
- TMB Reagent (One-Step): TMB11-1, 11 ml
- Stop Solution (1N HCl): SS11-1, 11 ml

Materials required but not provided:

- Precision pipettes and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-plate incubator/shaker with mixing speed of 150 rpm
- Plate washer
- Plate reader with an optical density range of 0-4 at 450nm
- Graph paper (PC graphing software is optional)

STORAGE

The kit should be stored at 4°C, and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase provided that the components are stored as described above.

^A The levels of rat anti-SRBC IgG are measured in nominal units and are calibrated with reference rat anti-SRBC serum at Life Diagnostics, Inc.

GENERAL INSTRUCTIONS AND PRECAUTIONS

1. Please read and understand the instructions thoroughly before using the kit.
2. All reagents should be allowed to reach room temperature (25°C) before use.
3. The assay was designed for use with serum or plasma obtained from rats ≥ 14 days after immunization with SRBC. Samples obtained prior to 14 days after immunization may contain high levels of anti-SRBC IgM that compete with anti-SRBC IgG for the immobilized SRBC antigens.
4. The optimal sample dilution should be determined empirically. However, studies performed at Life Diagnostics, Inc. suggest an initial sample dilution of 100-fold.
5. Serum or plasma samples must be diluted **at least** 25-fold in diluent.
6. Optimal results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.
7. Unlike other ELISA kits manufactured by Life Diagnostics, Inc., this assay typically has an elevated background signal as evidenced by an OD in the range of 0.4 – 1 OD units for the 6.25 u/ml standard. This does not detract from the performance of the assay.
8. Ant-SRBC IgG levels are undetectable in serum from naive animals.

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. Reconstitute the lyophilized rat anti-SRBC IgG standard stock with distilled or deionized water as described on the standard vial label (***the reconstituted standard should be aliquoted and frozen at -20°C after reconstitution if additional use is intended***).
2. Label 5 polypropylene or glass tubes as 100, 50, 25, 12.5 and 6.25 u/ml.
3. In the tube labeled 100 u/ml, prepare a 100 u/ml stock by mixing the volume of reconstituted standard stock with the volume of diluent detailed on the reference standard stock vial label.
4. Dispense 250 μ l of diluent into the tubes labeled 50, 25, 12.5 and 6.25 u/ml.
5. Prepare a 50 u/ml standard by diluting and mixing 250 μ l of the 100 u/ml standard with 250 μ l of diluent in the tube labeled 50 u/ml.
6. Similarly prepare the 25, 12.5 and 6.25 u/ml standards by serial dilution.

SAMPLE PREPARATION

Studies at Life Diagnostics, Inc. indicate that anti-SRBC IgG is present in serum or plasma from SRBC immunized rats at concentrations of approximately 2000 u/ml (14 days after

immunization). To obtain values within the range of the standard curve, we suggest that samples initially be diluted 100-fold using the following procedure:

1. For each test sample dispense 247.5 μ l of diluent into separate tubes.
2. Pipette and mix 2.5 μ l of the serum/plasma sample into a tube containing 247.5 μ l of diluent. This provides a 100-fold diluted sample.
3. Repeat this procedure for each sample to be tested.

Important: Do not use dilutions lower than 25-fold.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μ l of standards and diluted samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 45 minutes.
4. Aspirate the contents of the microtiter wells and wash the wells 5 times with 1x wash solution using a plate washer (400 μ l/well). The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.
6. Add 100 μ l of enzyme conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 45 minutes.
8. Wash as detailed in 4 to 5 above.
9. Dispense 100 μ l of TMB Reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 20 minutes.
11. Stop the reaction by adding 100 μ l of Stop Solution to each well.
12. Gently mix. *It is important to make sure that all the blue color changes to yellow.*
13. Read the optical density at 450 nm with a microtiter plate reader *within 5 minutes*.

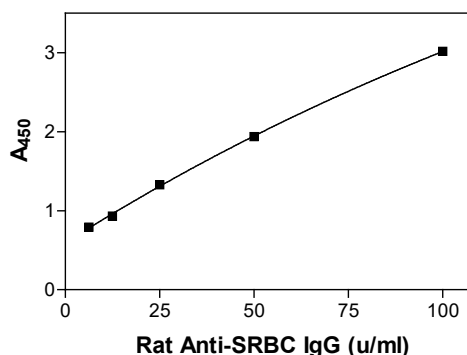
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in u/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of anti-SRBC IgG in u/ml from the standard curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of anti-SRBC IgG in the serum/plasma sample.
5. PC graphing software may be used for the above steps.
6. If the OD_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y-axis against anti-SRBC IgG concentrations on the X-axis 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.

Anti-SRBC IgG (u/ml)	A_{450}
100	3.016
50	1.939
25	1.330
12.5	0.933
6.25	0.793



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of and in accordance with the instructions detailed above.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

REFERENCES

1. GS Ladics. Use of SRBC antibody responses for immunotoxicity testing. *Methods* 41:9-19 (2007)
2. L Temple, TT Kawabata, AE Munson and KL White. Comparison of ELISA and Plaque-Forming Cell Assays for Measuring the Humoral Immune Response to SRBC in Rats and Mice Treated with Benzo[a]pyrene or Cyclophosphamide. *Fundamental and Applied Toxicology* 21(4):412-419 (1993)

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