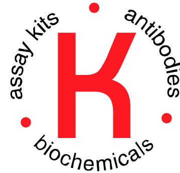

KAMIYA BIOMEDICAL COMPANY

Mouse Anti-Sheep Red Blood Cell (SRBC) IgG ELISA

For the quantitative determination of Anti-SRBC IgG in mouse serum and plasma

Cat. No. KT-574

For research use only, not for use in diagnostic procedures.

PRODUCT INFORMATION

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PRODUCT
The **K-ASSAY®** Mouse Anti-SRBC IgG ELISA is for the quantitative determination of Anti-SRBC IgG in mouse serum and plasma.

INTRODUCTION
Recent studies have demonstrated that suppression of anti-SRBC IgG levels by therapeutic agents serves as a useful indicator of immunosuppression. This ELISA allows rapid and quantitative measurement of mouse anti-SRBC IgG levels in serum or plasma samples.

PRINCIPLE
The mouse anti-SRBC IgG ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses detergent solubilized SRBC ghosts for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-mouse 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 and 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, and optical density is measured spectrophotometrically at 450 nm. The concentration of anti-SRBC IgG is proportional to the optical density of the test sample.

COMPONENTS
- SRBC coated 96-well plate (provided as 12 strips of 8 wells)
- Enzyme Conjugate Reagent: 11 mL
- Reference calibrator stock: lyophilized
- Diluent: 30 mL
- TMB Reagent (One-Step): 11 mL
- Stop Solution (1N HCl): 11 mL
- 20X Wash Solution: 50 mL

Materials required but not provided
- Plate reader with an optical density range of 0-4 at 450 nm
- Precision pipettes and tips
- Distilled or de-ionized water
- Graph paper (PC graphing software is optional)
- Absorbent paper or paper towels
- Polypropylene or glass tubes
- Vortex mixer
- Micro-Plate incubator/shaker mixing speed of ~150 rpm
- Plate washer

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 until the expiration date provided that the components are stored as described above.

GENERAL INSTRUCTIONS

1. Please read and understand the instructions thoroughly before using the kit.
2. The assay was designed for use with serum or plasma obtained from mice fourteen days after immunization with SRBC, at which point the immune response originates almost exclusively from IgG.
3. All reagents should be allowed to reach room temperature (18-25°C) before use.
4. The optimal sample dilution should be determined empirically. However, studies suggest an initial sample dilution of 50 fold.
5. Serum or plasma samples must be diluted **at least** 15-fold in diluent.
6. Optimum results are achieved if, at each step, reagents are pipetted into wells of the microtiter plate within 5 minutes.

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 of de-ionized water.

CALIRATOR PREPARATION

1. Reconstitute one vial of the lyophilized mouse anti-SRBC IgG calibrator stock with diluent as described on the calibrator vial label ***(the reconstituted calibrator should be aliquoted and frozen at -20°C after reconstitution if additional use is intended)***.
2. Label 5 polypropylene or glass tubes as 50, 25, 12.5, 6.25, and 3.125 u/mL.
3. Dispense 250 µL of diluent into the tubes labelled 50, 25, 12.5, 6.25, and 3.125 u/mL.
4. Prepare a 50 u/mL calibrator by diluting and mixing 250 µL of the reconstituted calibrator with 250 µL of diluent in the tube labelled 50 u/mL.
5. Similarly prepare the 25, 12.5, 6.25, and 3.125 u/mL calibrators by serial dilution.

SAMPLE PREPARATION

Note: Studies indicate that anti-SRBC IgG is present in mouse serum or plasma from SRBC immunized animals at concentrations in excess of 500 u/mL. In order to obtain values within the range of the calibration curve, we suggest that samples initially be diluted 50 fold using the following procedure for each sample tested:

1. For each test sample dispense 294 µL of diluent into separate tubes.
2. Pipette and mix 6 µL of the serum/plasma sample into a tube containing 294 µL of diluent. This provides a 50 fold diluted sample.
3. Repeat this procedure for each sample to be tested.

Important: Do not use dilutions lower than 15 fold.

PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of calibrators 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 (18-25°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 µL/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 µL of enzyme conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
8. Wash as detailed in 4 to 5 above.
9. Dispense 100 µL of TMB Reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
11. Stop the reaction by adding 100 µL 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

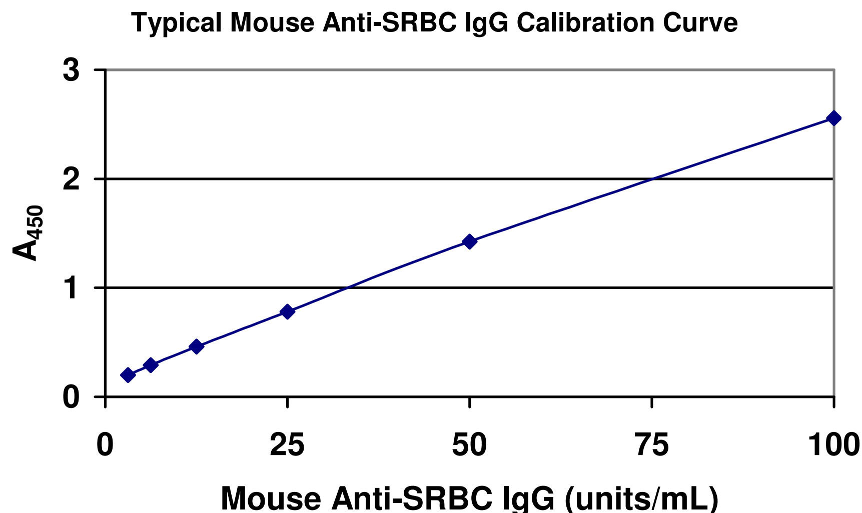
1. Calculate the average absorbance values (A_{450}) for each set of calibrators and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in u/mL on linear graph paper, with absorbance values on the vertical or Y-axis 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 calibration curve.

4. Multiply the derived concentrations by the dilution factor to determine the actual concentration for 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 calibration curve when tested at a dilution of 50, samples should be diluted appropriately and re-tested (do not use dilutions lower than 15 fold).

TYPICAL CALIBRATION CURVE

A typical calibration curve with optical density readings at 450 nm 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 calibration curve in each experiment.

Anti-SRBC IgG (u/mL)	Absorbance (450 nm)
100	2.556
50	1.423
25	0.782
12.5	0.461
6.25	0.292
3.125	0.201



LIMITATIONS OF THE PROCEDURE

- 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.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

FOR RESEARCH USE ONLY

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