

**KAMIYA BIOMEDICAL COMPANY**

# Mouse Anti-SRBC IgM ELISA

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

**Cat. No. KT-573**

**For research use only.**

## PRODUCT INFORMATION

### **Mouse Anti-SRBC IgM ELISA** **Cat. No. KT-573**

#### **PRODUCT**

The **K-ASSAY®** Mouse Anti-SRBC IgM ELISA is for the quantitative determination of Anti-SRBC IgM in mouse serum and plasma. For research use only.

#### **INTRODUCTION**

Recent studies have demonstrated that suppression of anti-SRBC (sheep red blood cell) IgM levels by therapeutic agents serves as a useful indicator of immunosuppression. This test kit allows rapid and quantitative measurement of mouse anti-SRBC IgM levels in serum or plasma samples.

#### **PRINCIPLE**

The Mouse Anti-SRBC IgM 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 IgM 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 IgM 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 IgM 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: 11 mL, 1N HCl
- Wash Buffer (20x): 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 with 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 five days after immunization with SRBC, at which point the immune response originates almost exclusively from IgM.
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 or de-ionized water.

## CALIBRATOR PREPARATION

1. Reconstitute the lyophilized mouse anti-SRBC IgM calibrator stock with diluent as described on the calibrator vial label. Then, dilute 346.7 µL of the reconstituted calibrator with 153.3 µL of diluent to give the 100 u/mL calibrator **(the reconstituted calibrator should be aliquoted and frozen at or below -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, and dispense 250 µL of diluent into each tube.
3. Prepare a 50 u/mL calibrator by diluting and mixing 250 µL of the 100 u/mL calibrator with 250 µL of diluent in the tube labelled 50 u/mL.
4. Similarly prepare the 25, 12.5, 6.25, and 3.125 u/mL calibrators by serial dilution.

## SAMPLE PREPARATION

**General Note: Studies indicate that anti-SRBC IgM is present in mouse serum or plasma from SRBC immunized animals at concentrations in excess of 2,000 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:**

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.**

## ASSAY 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 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

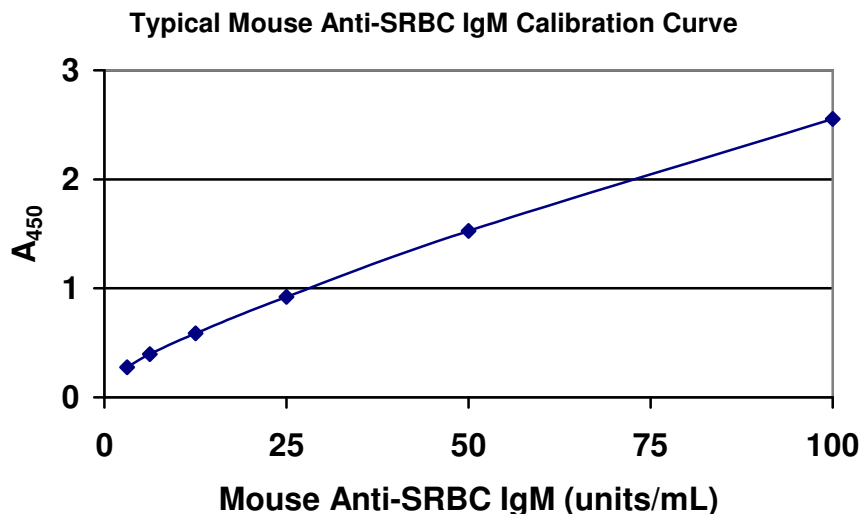
1. Calculate the average absorbance values ( $A_{450}$ ) for each set of reference calibrators and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each calibrator against its concentration in u/mL on linear graph paper, with absorbance values on the vertical or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

- Using the mean absorbance value for each sample, determine the corresponding concentration of anti-SRBC IgM in u/mL from the calibration curve.
- Multiply the derived concentrations by the dilution factor to determine the actual concentration for anti-SRBC IgM in the serum/plasma sample.
- PC graphing software may be used for the above steps.
- If the OD 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 IgM 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 IgM (u/mL)	A <sub>450</sub>
100	2.555
50	1.526
25	0.922
12.5	0.586
6.25	0.396
3.125	0.277



### 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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