KAMIYA BIOMEDICAL COMPANY

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

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

Cat. No. KT-572

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

PRODUCT INFORMATION

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PRODUCT

The **K-*ASSAY*®** Rat SRBC IgG ELISA is for the quantitative determination of SRBC IgG in rat serum or plasma.

PRINCIPLE

The Rat 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-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 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

- Microtiter Plate: SRBC coated 96-well plate (12 strips of 8 wells)
- Enzyme Conjugate Solution: 11 mL
- Calibrator: Lyoph.
- Diluent Buffer: 30 mL
- TMB Solution: 11 mL
- Stop Solution: 11 mL, 1N HCl
- Wash Buffer (20x): 50 mL

Materials or Equipment required but not provided

- Plate reader (450 nm)
- Micropipette and tips
- De-ionized water
- Graph paper (PC software is optional)
- Paper towels
- Polypropylene or glass tubes
- Vortex mixer
- Plate shaker/incubator
- Plate washer

STORAGE

Store at 4℃. Microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. The kit is stable until the expiration date when stored as noted in this section.

General Instructions

1. Please read and understand the instructions thoroughly before using the kit.
2. This kit is designed to measure anti-SRBC IgG levels in Rat serum or plasma collected 14 days after immunization with SRBC. Samples obtained prior to 14 days after immunization with SRBC may contain high levels of anti-SRBP IgM that compete with anti-SRBP IgG for the immobilized SRBC antigens.
3. All reagents should be allowed to reach room temperature (18-25℃) before use.
4. The optimal sample dilution should be determined empirically. However, studies suggest an initial sample dilution of 100 fold.
5. Serum and plasma samples must be diluted at least 25 fold in diluent.
6. Optimum results are achieved if, at each step, reagents are pipetted into wells of the microtiter plate within 5 minutes.

7. Unlike other assays manufactured by Kamiya Biomedical Company 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 calibrator. This does not detract from the performance of the assay.
8. Anti-SRBC IgG levels are undetectable in serum from native animals.

PREPARATION OF REAGENTS

Wash Buffer
The wash solution is provided as 20x stock. Prior to use dilute the contents of the bottle (50 mL) with 950 mL of distilled of deionized water.

Calibrator
1. The Rat anti-SRBC IgG calibrator is provided as lyophilized stock. Reconstitute with volume of diluent indicated on the vial label. The reconstituted calibrator is stable at 4℃ for one week but should be aliquoted and stored frozen at -20℃ after reconstitution if future use is intended.
2. Label 5 polypropylene or glass tubes as 100, 50, 25, 12.5, and 6.25 u/mL.
3. Into the tube labeled 100 u/mL prepare a 100 u/mL stock by mixing the volume of reconstituted calibrator stock with the volume of diluent detailed on the vial label.
4. Dispense 250 µL of diluent into the tubes labelled 50, 25, 12.5, and 6.25 u/mL.
5. 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.
6. 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 rat serum or plasma at concentrations of ~ 2,000 u/mL (14 days after immunization). In order to obtain values within range of the calibration curve, we suggest samples initially be diluted 100 fold using the following procedure for each sample tested. Optimal dilutions may need to be determined empirically.
1. For each test sample dispense 247.5 µL of diluent into separate tubes.
2. Pipette and mix 2.5 µL of the serum/plasma sample into the tube containing 247.5 µL of diluent. This provides a 100 fold diluted sample.
3. Repeat this procedure for each sample to be tested.
4. Do not use dilutions lower than 25 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℃) 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℃) for 45 minutes.
8. Wash as detailed in 4 and 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 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 for each set of 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.
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 values of samples fall outside the calibration curve when tested at a dilution of 100, samples should be diluted appropriately and re-tested.

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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