KAMIYA BIOMEDICAL COMPANY

Rat IgG2a ELISA

For the quantitative determination of IgG2a in rat serum or plasma

Rat. No. KT-753

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The **K-ASSAY®** Rat IgG2a ELISA is an enzyme immunoassay for the quantitative determination of IgG2a in rat serum or plasma. For research use only.

INTRODUCTION
Four subclasses of IgG are present in rat: IgG1, IgG2a, IgG2b and IgG2c. Respective concentrations in 80-day old Lewis rats were found to be 0.10, 0.95, 2.06 and 0.09 mg/mL. Levels of the different subclasses vary with age and in response to immune stimulus.

The rat IgG2a ELISA kit is designed for measurement of IgG2a in rat serum or plasma. The assay uses mouse monoclonal anti-rat IgG2a for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated mouse monoclonal anti-rat IgG2a antibodies for detection. When used as directed, the kit recognizes only IgG2a in rat serum. It does not recognize mouse IgG. Crossreactivity with immunoglobulins from other species has not been investigated.

PRINCIPLE
Test samples are diluted and incubated in the anti-rat IgG2a coated microtiter wells for 45 minutes alongside rat IgG2a calibrators. The microtiter wells are subsequently washed, and anti-rat IgG2a-HRP conjugate is added and incubated for 45 minutes. IgG2a molecules are thus sandwiched between the immobilization and detection antibodies. 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 IgG2a is proportional to the optical density of the test sample and is derived from a calibration curve.

COMPONENTS
- Anti-Rat IgG2a Coated 96-well Plate (12 strips of 8 wells)
- Anti-Rat IgG HRP Conjugate Reagent, 11 mL
- Reference Calibrator (lyophilized)
- Wash Buffer (20X), 50 mL
- Diluent, 50 mL
- TMB Reagent (One-Step), 11 mL
- Stop Solution (1N HCl), 11 mL

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

GENERAL INSTRUCTIONS
All reagents should be allowed to reach room temperature (18-25℃) before use.
Please take the time to completely read and understand this kit insert before starting your assay. Don't hesitate to contact us

by telephone or e-mail should you require technical assistance or clarification.
Optimal results are achieved if, at each step, reagents are pipetted into the 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.

HRP CONJUGATE PREPARATION

The HRP Conjugate must be diluted with 3 volumes of diluent shortly before use. For each 8-well strip mix 0.250 mL of HRP conjugate with 0.75 mL of diluent.

SAMPLE PREPARATION

General Note: IgG2a is typically present in rat serum or plasma at concentrations of ~2 mg/mL. In order to obtain values within range of the calibration curve, we suggest that samples initially be diluted 10,000 fold using the following procedure for each sample to be tested:

1. Dispense 198 µL and 247.5 µL of diluent into separate tubes.
2. Pipette and mix 2 µL of the serum/plasma sample into the tube containing 198 µL of diluent. This provides a 100 fold diluted sample.
3. Mix 2.5 µL of the 100 fold diluted sample with the 247.5 µL of diluent in the second tube. This provides a 10,000 fold dilution of the sample.
4. Repeat this procedure for each sample to be tested.

In order to avoid matrix effects, serum dilutions less than 2,000 fold (i.e., 1,000 fold) should be avoided. Tissue extracts and body fluids other than serum or plasma will likely have lower IgG2a levels than those found in serum. Optimal dilutions of such samples should be determined empirically.

CALIBRATOR PREPARATION

1. The rat IgG2a calibrator is provided as a lyophilized stock. Reconstitute with 1.0 mL of distilled or deionized water ***(the reconstituted calibrator is stable at 4^{o}C for one day but should be aliquoted and frozen at -20^{o}C after reconstitution if future use is intended)***.
2. Label 6 polypropylene or glass tubes as 250, 125, 62.5, 31.25, 15.63 and 7.81 ng/mL.
3. Into the tube labeled 250 ng/mL, pipette the volume of diluent detailed on the IgG2a calibrator vial label. Then add the indicated volume of IgG2a calibrator and mix gently. This provides the 250 ng/mL calibrator.
4. Dispense 250 µL of diluent into the tubes labeled 125, 62.5, 31.25, 15.63, 7.81 ng/mL.
5. Prepare a 125 ng/mL calibrator by diluting and mixing 250 µL of the 250 ng/mL calibrator with 250 µL of diluent in the tube labeled 125 ng/mL.
6. Similarly prepare the 62.5, 31.25, 15.63 and 7.81 ng/mL calibrators by serial dilution.

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 (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 HRP 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 µL 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 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 reference calibrators and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its

concentration in ng/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 IgG2a in ng/mL from the calibration curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of IgG2a in the sample.
5. If available, PC graphing software should be used for the above steps. We find that a good fit of the data is obtained with a one site, total and nonspecific binding model.
6. If the A_{450} values of samples fall outside the range of the calibration curve samples should be re-diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A representative calibration curve with optical density readings at 450 nm on the Y-axis against IgG2a concentration 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.

IgG2a (ng/mL)	Absorbance (450 nm)
250	3.278
125	2.019
62.5	1.195
31.25	0.791
15.63	0.555
7.81	0.451

STORAGE

The kit will remain stable until the expiration date provided that the components are stored at 4℃. It is important that the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. 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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