



KAMIYA BIOMEDICAL COMPANY

Rat Anti-Polyethylene Glycol (PEG) IgG ELISA

**For the quantitative determination of anti-PEG IgG
in rat serum and plasma.**

Cat. No. KT-679

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT

The **K-ASSAY®** Rat Anti-Polyethylene Glycol (PEG) IgG ELISA is an enzyme immunoassay for the quantitative determination of anti-PEG IgG in rat serum and plasma. For research use only.

INTRODUCTION

The attachment of polyethylene glycol chains to therapeutic biologic agents, a process referred to as PEGylation, prolongs the circulating half-life of the modified protein by slowing proteolytic degradation and by masking it from the immune system. However, repeated injections of PEGylated proteins can induce anti-PEG antibodies that increase the rate of clearance and thereby decrease efficacy (accelerated blood clearance, or ABC, phenomenon). In order to aid research in this important area, we have developed a rat anti-PEG IgG ELISA kit.

PRINCIPLE

The **K-ASSAY®** Rat anti-PEG IgG ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses immobilized mono mPEGylated BSA (20 kDa PEG chain) as the capture antigen (coated on the microtiter wells) and horseradish peroxidase (HRP) conjugated anti-rat IgG antibodies for detection. Serum or plasma samples are diluted and incubated alongside calibrators in the microtiter wells for 1 hour. The wells are subsequently washed, and HRP conjugate is added and incubated for 45 minutes. Anti-PEG IgG molecules are thus sandwiched between immobilized PEG 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-PEG IgG is proportional to the absorbance at 450 nm and is derived from a calibration curve.

This assay primarily detects antibodies directed against the polyoxyethylene backbone of PEG. Our studies in mice and rabbits have demonstrated that the majority of anti-PEG antibodies induced by immunization with PEGylated proteins are directed against the PEG backbone.

COMPONENTS

- PEG-BSA Coated 96-well Plate (provided as 12 strips of 8 wells). **Store at -20°C.**
- Anti-Rat IgG HRP Conjugate Stock, 50 µL. **Store at -20°C.**
- Reference Calibrator (lyophilized), 1 vial. **Store at -20°C.**
- PEG Diluent, 50 mL
- PEG-HRP Wash Solution (20X), 50 mL
- TMB Reagent, 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
- A microtiter plate reader capable of measuring absorbance at 450 nm with a range of 0-4 OD
- Graph paper (PC graphing software is optional)

- Plate washer

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Please read and understand the instructions thoroughly before using the kit.
3. **This kit is designed to measure anti-PEG IgG levels in serum collected ≥ 14 days after immunization with PEG.** Serum collected at post-immunization times less than 14 days may contain high levels of anti-PEG IgM that compete with anti-PEG IgG for the immobilized PEG, thereby causing interference.
4. The optimal sample dilution should be determined empirically. However, our studies suggest an initial sample dilution of 500 fold. The kit was validated using serum dilutions ≥ 50 fold.
5. 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.

CALIBRATOR PREPARATION

1. The rat anti-PEG IgG calibrator is provided as a lyophilized stock. Reconstitute the stock as described on the vial label.
2. Label 6 polypropylene or glass tubes as 100, 50, 25, 12.5 and 6.25 u/mL.
3. In the tube labeled 100 u/mL, prepare the 100 u/mL calibrator as detailed on the stock vial label.
4. Dispense 350 μ L of diluent into the remaining tubes.
5. Prepare a 50 u/mL calibrator by diluting and mixing 350 μ L of the 100 u/mL calibrator with 350 μ L of diluent in the tube labeled 50 u/mL.
6. Similarly prepare the 25, 12.5 and 6.25 u/mL calibrators by serial dilution.

SAMPLE PREPARATION

General Note: Our studies indicate that anti-PEG IgG is undetectable in serum from control rats. However, in serum from PEG-KLH immunized rats, levels of 1,544 to 19,994 u/mL ($11,726 \pm 6,636$, mean \pm SD, n = 8) were observed 25 days after immunization. Levels will vary with the immunization protocol and the PEG carrier protein used. We **suggest** that samples initially be diluted 2,000 fold using the following procedure for each sample to be tested but optimal dilutions must be determined empirically. A 2,000 fold dilution may be achieved as follows:

1. Dispense 48 μ L and 332.5 μ L of diluent into separate tubes.
2. Pipette and mix 2 μ L of the serum/plasma sample into the tube containing 48 μ L of diluent. This provides a 25 fold diluted sample.
3. Mix 17.5 μ L of the 25 fold diluted sample with the 332.5 μ L of diluent in the second tube. This provides a 500 fold dilution of the sample.
4. Repeat this procedure for each sample to be tested.

HRP CONJUGATE PREPARATION

Approximately 5 minutes before needed, dilute the HRP Conjugate stock with diluent (equilibrated to room temperature) as directed on the vial label.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μ L of calibrators and diluted samples into the wells (we recommend that samples be tested in triplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 1 hour.
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 adsorbent paper or paper towels to remove all residual wash buffer.
6. Add 100 μ L of diluted HRP conjugate 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 μ L 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 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 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 anti-PEG IgG in u/mL from the calibration curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of anti-PEG IgG in the serum/plasma sample.
5. PC graphing software may be used for the above steps.
6. If the OD₄₅₀ values of samples fall outside the calibration curve when tested at the suggested dilution of 500, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with optical density readings at 450 nm on the Y axis against anti-PEG 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.

Anti-PEG IgG (u/mL)	Absorbance (450 nm)
100	2.635
50	1.351
25	0.660
12.5	0.311
6.25	0.182

STORAGE

The reference calibrator, HRP conjugate and the PEG-BSA coated plate should be stored at -20 °C. All remaining kit components should be stored at 4 °C. 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.

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.
3. Kits are validated using shaking incubators set at 150 rpm and 25 °C. Performance of the assay at lower temperatures and/or mixing speeds will likely result in lower absorbance values.
4. Use only the wash buffer and dilution buffer provided with the kit. PEG and PEGylated compounds are found in many buffers conventionally used in ELISA's and cannot be used with this kit.

FOR RESEARCH USE ONLY

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