KAMIYA BIOMEDICAL COMPANY

Rabbit Anti-PEG IgM ELISA

For the quantitative determination of anti-PEG IgM in rabbit serum or plasma

Cat. No. KT-1903

For Research Use Only.

PRODUCT INFORMATION
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PRODUCT
The **K-ASSAY®** Rabbit Anti-PEG IgM ELISA is an enzyme immunoassay for the quantitative determination of anti-PEG IgM in rabbit serum or plasma. For research use only.

INTRODUCTION
Attachment of polyethylene glycol (PEG) 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, it has been reported that repeated injections of PEGylated proteins can induce anti-PEG antibodies that increase the rate of clearance and decrease drug efficacy (accelerated blood clearance, or ABC phenomenon). To aid research in this important area, we have developed a rabbit anti-PEG IgM ELISA kit.

PRINCIPLE
The assay uses immobilized mono mPEGylated BSA (20 kDa PEG chain) as the capture antigen (coated on microtiter wells) and horseradish peroxidase (HRP) conjugated anti-rabbit IgM for detection. Serum or plasma samples are diluted and incubated alongside calibrators in the microtiter wells for 45 minutes. The wells are subsequently washed, and HRP conjugate is added and incubated for 45 minutes. Anti-PEG IgM molecules are sandwiched between immobilized PEG and the detection antibody conjugate. 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 anti-PEG IgM 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.

COMPONENTS
- PEG-BSA coated plate (12 x 8-wells) **Store at -20℃**
- Anti-IgM HRP Stock **Store at -20℃**
- Anti-PEG IgM Stock Calibrator (lyophilized) **Store at -20℃**
- 20x HRP PEG Wash, 50 mL
- HRP PEG Diluent, 50 mL
- TMB, 11 mL
- Stop Solution, 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Pipettors and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm.
- Curve fitting software

STORAGE
The reference stock, HRP conjugate and the PEG-BSA coated plate should be stored at -20℃. All remaining kit components should be stored at 4℃. The microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable until the expiration date provided that the components are stored as described.

GENERAL INSTRUCTIONS

1. Please read and instructions thoroughly before using the kit.
2. All reagents should be allowed to reach room temperature (25°C) before use.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Use only the wash solution 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.
5. Kits are validated using plate shakers 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.
6. 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 anti-PEG IgM calibrator is provided as a lyophilized stock. Reconstitute as described on the vial label to obtain the 100 u/mL calibrator.
2. Label 7 polypropylene microcentrifuge tubes as 50, 25, 12.5, 6.25, 3.125, 1.563 and 0 u/mL.
3. Dispense 250 µL of diluent into the tubes.
4. 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 labeled 50 u/mL.
5. Similarly prepare the 25, 12.5, 6.25, 3.125 and 1.563 u/mL calibrators by serial dilution.

Unused reconstituted stock should be stored frozen at or below -20°C if future use is intended.

SAMPLE PREPARATION

In studies, we found anti-PEG IgM levels ranging from approximately 1,000 u/mL in naïve serum to 350,000 u/mL in serum from rabbits injected with PEG-KLH. Optimal dilutions must be determined empirically. However, we suggest testing each sample at dilutions of 500- and 5,000-fold. It is important that the diluent provided with the kit be used for dilution. Do not substitute other buffers.

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 µL of calibrators and diluted samples into the wells (we recommend testing in duplicate).
3. Incubate on a plate shaker at 150 rpm/25°C for 45 minutes.
4. Aspirate the contents of the microtiter wells and wash the wells five times with 1x wash solution using a plate washer (400 µL/well).
5. Strike the wells sharply onto absorbent paper to remove all residual wash solution.
6. Add 100 µL of diluted HRP conjugate into each well.
7. Incubate on a plate shaker at 150 rpm/25°C for 45-minutes.
8. Wash as detailed above.
9. Dispense 100 µL of TMB into each well.
10. Incubate on a plate shaker at 150 rpm/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 five minutes.

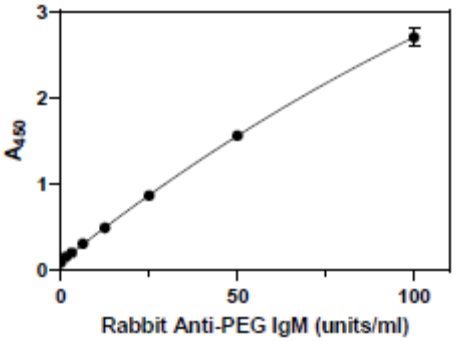
CALCULATION OF RESULTS

1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus concentration.
2. Fit the calibration curve to an appropriate model (we fit to two-site, total and non-specific binding model) and determine concentration of the diluted samples from the calibration curve.
3. Multiply the derived concentration by the dilution factor to determine concentration in the original samples.
4. If the A_{450} values of samples fall outside the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

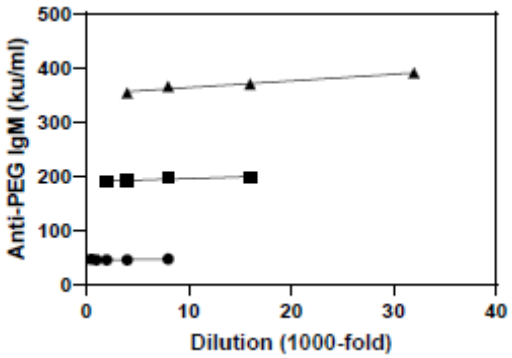
A typical calibration curve with optical density readings at 450 nm on the Y-axis against anti-PEG IgM concentrations on the X-axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns.

Anti-PEG IgM (u/ml)	A_{450}
100	2.709
50	1.563
25	0.866
12.5	0.492
6.25	0.305
3.125	0.201
1.563	0.155
0	0.086



ASSAY PERFORMANCE

Parallelism: To assess performance of the assay, three samples containing anti-PEG IgM at concentrations of 44,886, 193,233 and 368,990 u/mL were serially diluted to produce values within the dynamic range of the assay.



ASSAY UNITS

We have worked with PEG antibodies from mice, rats, monkeys, rabbits and humans. Excepting mouse monoclonal antibodies that can be purified under gentle conditions, it has been our experience that elution of PEG antibodies from affinity columns causes significant and indeterminate inactivation. It is therefore very difficult to prepare and quantitate pure functional PEG IgM and IgM for calibration purposes. For this reason, we decided to use nominal units for measurement. All batches of anti-PEG stock are calibrated to reference serum stored at Kamiya Biomedical Company.

FOR RESEARCH USE ONLY

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