



Rabbit Anti-PEG IgM ELISA kit (MAK-0017)

This product is for research use only and is not intended for diagnostic use.

PRODUCT INFORMATION

Cat No. MAK-0017

Storage Indirect ELISA

Principle This kit is for research use only. Under no circumstances should it be used for therapeutic or human diagnostic applications.

Intended Use 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.

Introduction 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 standards 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 standard curve.

This assay primarily detects antibodies directed against the polyoxyethylene backbone of PEG.

Principle of the assay

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 anti-PEG ELISA kit. Used for new drug research and development

Storage Note

The reference stock, 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. Kits will remain stable for six months from the date of purchase provided that the components are stored as described.

Materials

Materials provided with the kit:

- PEG-BSA coated plate (12 x 8-wells)
 - Anti-Rabbit IgM HRP Stock (lyophilized)
 - Reference Stock1 (lyophilized)
 - 20x HRP PEG Wash: PEGW50-20, 50 ml
 - HRP PEG Diluent: PEGD50-1, 50 ml
 - TMB: TMB11-1, 11 ml
 - Stop Solution: SS11-1, 11 ml
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General instructions

1. Please read and understand the 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.
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Diluent

The diluent (PEGD50-1) is formulated for measurement PEG antibodies. It is supplied ready to use. DO NOT substitute other buffers.

Sample Preparation

In studies at Mabioway Co., Ltd., we found that anti-PEG IgM levels in human samples ranged from undetectable to >23,000 u/ml. Optimal dilutions must be determined empirically. However, we suggest that samples initially be tested at 200-fold dilutions.

HRP Anti-PEG Conjugate

Approximately 15 minutes before needed, reconstitute the lyophilized HRP conjugate as described on the vial label and mix gently. Dilute as described on the vial label to give the working conjugate solution. The reconstituted conjugate stock must be stored at -20°C in a sealed vial if future use is intended.

Wash Solution

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 deionized water.

Standards

- 1.The anti-PEG IgM standard is provided as a lyophilized stock. Reconstitute as described on the vial label to obtain the 100 u/ml standard.
- 2.Label 7 polypropylene microcentrifuge tubes as 50, 25, 12.5, 6.25, 3.125, 1.563 and 0 u/ml.
- 3.Dispense 250 ul of diluent into the tubes.
- 4.Prepare a 50 u/ml standard by diluting and mixing 250 ul of the 100 u/ml standard with 250 ul 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 standards by serial dilution.

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

Procedure

- 1.Secure the desired number of coated wells in the holder.
- 2.Dispense 100 ul of standards 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 ul/well).
- 5.Strike the wells sharply onto absorbent paper to remove all residual wash solution.
- 6.Add 100 ul 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 ul 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 ul 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 minutes².
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Important Tips

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Results

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus concentration.
 2. Fit the standard 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 standard curve.
 3. Multiply the derived concentration by the dilution factor to determine concentration in the original samples.
 4. If the A₄₅₀ values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.
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