Sandwich ELISA for detection of PEGylated proteins kit (MAK-0008)

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

PRODUCT INFORMATION

Cat No.	MAK-0008
Storage	Sandwich ELISA
Principle	This kit is for research use only. Under no circumstances should it be used for therapeutic or 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. The pharmacodynamics of PEGylated proteins are often evaluated using specific assays for the protein itself. That approach often requires the time consuming and expensive development of an ELISA for the protein of interest. The PEG backbone ELISA allows measurement of the PEG portion of the PEGylated protein and is therefore suitable for assessment of the pharmacodynamics of a range of PEGylated proteins. As shown in the figure below, sensitivity of the assay varies with chain-length and the extent of PEGylation. To determine PEG concentration in unknown samples, the end-user must establish a standard curve using the PEG reagent under investigation.
Introduction	The assay uses two different monoclonal antibodies that recognize the PEG backbone. One is coated on wells of the microtiter plate and is used for capture; the other is conjugated to horseradish peroxidase (HRP) and is used for detection. Serum samples are diluted at least 10-fold in the provided dilution buffer and incubated alongside reference standards1 in the microtiter wells for 45-minutes. The wells are subsequently washed. HRP conjugate is added and incubated for 45 minutes. If present, PEG molecules are sandwiched between

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the capture and detection antibodies. The wells are then washed to remove unbound HRP-conjugate. TMB is added and incubated for 20 minutes. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow. Absorbance is measured at 450 nm. The concentrations of PEG in the samples are derived from a standard curve.

Principle of the assay

PEGylation of biologics prolongs their half-life by slowing proteolytic degradation and decreasing the rate of clearance from the circulatory system. The pharmacodynamics of PEGylated proteins are often evaluated using specific assays for the protein itself. That approach often requires the time consuming and expensive construction of an ELISA for the protein of interest. The PEG ELISA manufactured by Mabioway Co., Ltd. allows measurement of the PEG portion of the PEGylated protein and is therefore suitable for assessment of the pharmacodynamics of a range of PEGylated proteins.

Storage Note

The test stock, HRP conjugate, and the antibody 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:

- Anti-PEG coated plate (12 x 8-wells)
- Anti-PEG HRP Stock (lyophilized)
- Test Stock (lyophilized)
- 20x HRP PEG Wash: PEGW50-20, 50 ml
- PEG Diluent: PEGBC50-1, 50 ml
- TMB: TMB11-1, 11 ml
- Stop Solution: SS11-1, 11 ml

General instructions

Please fully read and thoroughly understand the instructions before using the kit.

1.All reagents should be allowed to reach room temperature (25°C) before use.

2.The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

3.Use only the wash solution and dilution buffers provided with the kit. They are specially formulated for measurement of PEG.

4.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 result in lower absorbance values.

5.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 (PEGBC50-1) is formulated for measurement PEG. It is supplied ready to use. DO NOT substitute other buffers.
Sample Preparation	In studies at Mabioway Co., Ltd., we performed spike-recovery experiments using rabbit serum. When serum was spiked with 250 or 50 ng/ml of 20 kDa mono mPEG-BSA, respective recoveries of 244 and 39 ng/ml were obtained. Dilutional linearity was observed. To avoid matrix effects serum must be diluted at least ten-fold in diluent PEGBC50-1. The end user must determine optimal dilutions.
HRP Anti-PEG Conjugate	Approximately 15 minutes before needed, reconstitute the lyophilized HRP conjugate as directed on the vial label and mix gently. Then, dilute as described on the vial label to give the working conjugate solution. The reconstituted conjugate stock should be stored at or below -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. DO NOT substitute other wash solutions.
Standards	The Test Stock provided with the kit consists of BSA to which a single chain of 20 kDa mPEG is attached. It is provided for optional use to confirm that the assay is working as intended. As described in the next section, the end user must establish a standard curve using the PEG reagent under investigation. 1.Reconstitute the Test Stock as described on the vial label. 2.Label 8 microcentrifuge tubes2 as 50, 16.67, 5.56, 1.852, 0.617, 0.206, 0.069, and 0.023 ng/ml. 3.In the tube labeled 50 ng/ml prepare the 50 ng/ml standard as detailed on the stock vial label. 4.Dispense 250 □l of diluent into the remaining tubes. 5.Prepare a 16.67 ng/ml standard by diluting and mixing 125 □l of the 50 ng/ml standard with 250 □l of diluent in the tube labeled 16.67 ng/ml. 6.Similarly prepare the remaining standards by three-fold serial dilution.
Procedure	1.Secure the desired number of coated wells in the holder. 2.Dispense 100 µL of standards and diluted samples into the wells (we

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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, if necessary, 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 minutes3.

Important Tips /

Results

1.Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus log10 of the concentration.
2.Fit the standard curve to a four-parameter logistic regression (4PL) equation (x axis = log10 concentration) and determine the concentration of the samples from the standard curve (remember to derive the antilog).
3.Multiply the derived concentration by the dilution factor to determine the actual concentration in the 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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