Competitive ELISA for detection of PEGylated proteins kit (MAK-0007)

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

PRODUCT INFORMATION

Cat No.	MAK-0007
Storage	Competitive ELISA
Principle	This kit is for research use only. Under no circumstances should it be used for therapeutic or diagnostic applications.
Intended Use	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.
Introduction	The format of the assay is a direct competitive ELISA.
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

Upon receipt the HRP Anti-PEG vial, the PEG BSA standard and the PEG coated 96 well plate should be placed in a -20°C freezer until use. Do not store at lower temperatures. The remainder of the kit should be stored in a refrigerator at 2-8°C.

Materials

Materials provided with the kit:
• PEG Coated 96-well Microtiter Plate (12 detachable strips of 8 wells). Store at -20°C
• HRP anti-PEG Conjugate, 1 vial. Store at -20°C.
• HRP PEG Diluent: PEGD50-1, 50 ml
• PEG-BSA Standard, 1 vial.* Store at -20°C.
• HRP PEG Wash Buffer: PEGW50-20, 50 ml
• TMB Reagent: 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.

Diluent

The diluent (PEGBC50-1) is formulated for measurement PEG. It is supplied ready to use. DO NOT substitute other buffers.

Sample Preparation

The concentration of PEGylated protein in serum or plasma depends on several factors: the route of injection, the amount injected, and the time after injection at which serum or plasma is collected. Because such variables are user defined, optimum dilution must be determined empirically.

HRP Anti-PEG Conjugate

Determine the volume of conjugate required (0.5 ml per 8-well strip) and dilute

	the HRP anti-PEG conjugate stock with diluent as described on the vial label. Prepare shortly before use.
Wash Solution	The wash solution is provided as a 20x stock. Dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.
Standards	1. Label 8 polypropylene or glass tubes as 1000, 200, 40, 8, 1.6, 0.32, 0.064 and 0 ng/ml. 2. Prepare the 1000 ng/ml standard as described on the PEG standard vial label. 3. Dispense 200 µl of diluent into the tubes labeled 200, 40, 8, 1.6, 0.32, 0.064 and 0 ng/ml. 4.Pipette 50 µl of the 1000 ng/ml PEG standard into the tube labeled 200 ng/ml and mix. This provides the working 200 ng/ml PEG-BSA standard. 5.Similarly prepare 40, 8, 1.6, 0.32, and 0.064 ng/ml standards by five-fold serial dilution.
Procedure	1.Secure the desired number of coated wells in the holder. 2.Dispense 50 µl of standards and samples into the wells (we recommend that standards and samples be tested in triplicate). 3.Add 50 µl of HRP anti-PEG conjugate into each well. 4.Incubate on an orbital micro-plate shaker at 150 rpm at room temperature (25°C) for 1 hour. 5.Using a plate washer, wash the wells six times with 400 µl of wash buffer per well. 6.Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets. 7.Dispense 100 µl of TMB reagent into each well. 8.Gently mix on an orbital micro-plate shaker at 150 rpm for 20 minutes. 9.Stop the reaction by adding 100 µl of Stop Solution to each well. 10. Gently mix until all the blue color changes to yellow. 11. Read absorbance at 450 nm with a plate reader within 5 minutes.
Important Tips	• All reagents must be allowed to reach room temperature (25°C) before use. • ALWAYS add samples and standards to the microtiter wells before adding the HRP anti-PEG conjugate. • Do not substitute user-prepared buffers for those provided with the kit (i.e., diluent or wash buffer). Many commercially available materials contain PEG or PEGylated molecules, and this will affect performance of the kit. For this reason, care should be taken when selecting other components used in studies of PEGylated proteins.

• Tween-20 is a polyoxyethylene containing detergent commonly used in ELISA dilution and wash buffers. It will interfere with this ELISA. The same is true for other polyoxyethylene detergents.
• Do not add azide as a preservative to serum or plasma samples. Azide is an inhibitor of HRP and will invalidate the assay.
• We strongly recommend that a plate washer be used to wash the microtiter wells. Only use the wash buffer provided with the kit. Wash all tubing and vessels of the plate washer with distilled or deionized water prior to use and thoroughly prime the plate washer with wash buffer.
• When preparing standards, we routinely perform appropriate serial dilutions in a blank 96-well polystyrene plate using a multipipettor. Samples are also prepared and/or dispensed into the blank 96-well plate in the layout to be used in the ELISA. This allows for quick and easy transfer of samples and standards to the ELISA plate using a multipipettor.
• If a standard other than that provided with the kit is to be used, we suggest that the standard curve range be determined using 10-fold dilutions, starting with a concentration of 10 µg/ml. Using a single 8-well strip, this allows a concentration range from 0.1 ng/ml to 10 µg/ml to be inexpensively investigated. The useful standard curve range can then be fine-tuned.

Results

PC graphing software should be used to calculate results.
1. Calculate the average absorbance values (A450) for each set of reference standards and samples.
2.Construct a standard curve by plotting the mean absorbance obtained from each reference standard against the log10 of its concentration in ng/ml, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3.Fit the data to a sigmoidal dose response (variable slope) model. The upper limit of the curve may be fixed at the value defined by the 0 ng/ml “standard” and the lower limit of the curve may be fixed at the value of the 1000 ng/ml kit standard.
4.Using the mean absorbance value for each sample, determine the corresponding log10 concentration of PEGylated protein from the standard curve and derive the concentration in ng/ml by calculating the anti-log10.
5.We strongly recommend that only absorbance values of samples falling within the middle 50% region of the standard curve be used to determine PEG concentrations. For example, if the absorbance values of the low (zero ng/ml) and high standards are 1.6 and 0.1, only use sample absorbance values falling within the 1.225 and 0.475 range.
6.Multiply the derived concentration by the dilution factor to determine the actual concentration of PEGylated protein in the serum/plasma sample.
7.If the A450 values of samples fall outside the useful range of the standard curve, samples should be diluted appropriately and re-tested.