Instructions for the use of the MeO-(PEG)$_n$-NHS

Introduction

The BroadPharm MeO-(PEG)$_n$-NHS ester reagents enable simple and efficient modification of proteins and other molecules that have primary amines. Modification results in the addition of polyethylene glycol (PEG) spacers (pegylation) with terminal methyl groups. The PEG spacer is hydrophilic (water-soluble), and this property is transferred to the labeled macromolecule. Consequently, pegylation of proteins and peptides can significantly increase water solubility and reduce aggregation, often without adversely affecting their biological activities. Pegylation can also reduce immunogenicity of the labeled molecule. The branched, trimethylsuccinimidyl (TMS) reagent can efficiently cover the surface of a protein to impart these stabilizing properties even where few modification sites exist on the protein. Typical pegylation reagents contain heterogeneous mixtures of different PEG chain lengths; however, Broadpharm Pegylation Reagents are homogeneous compounds of defined molecular weight and spacer arm length, providing precision in optimizing modification applications. *N*-Hydroxysuccinimide (NHS) esters are the most popular type of reactive group used for protein modification. In pH 7-9 buffers, NHS-ester reagents react efficiently with primary amino groups ($-NH_2$) by nucleophilic attack, forming amide bonds and releasing the NHS. Proteins typically have many sites for labeling, including the primary amine in the side chain of each lysine (K) residue and the N-terminus of each polypeptide. The MeO-(PEG)$_n$-NHS ester reagents are readily soluble in water or organic solvents such as DMSO, methylene chloride and DMF.

Product Information

Storage: Upon receipt store desiccated at -20°C.

• The MeO-(PEG)$_n$-NHS ester reagents are viscous pale liquids that are difficult to weigh and dispense. To facilitate handling, make a stock solution by dissolving the reagent in a dry (anhydrous, molecular-sieve treated) organic solvent, such as dimethylformamide (DMF) and dimethylsulfoxide (DMSO). Minimize reagent exposure to moisture because the NHS-ester reactive group is susceptible to hydrolysis. Store unused stock solution in a moisture-free condition (e.g., capped under an inert gas such as argon or nitrogen) at -20°C. Equilibrate reagent vial to room temperature before opening to avoid moisture condensation inside the container. Minimize exposure to air by keeping the stock solution capped by a septum through which reagent can be removed with a syringe. With proper handling, the stock solution is stable for three months.

• Avoid buffers containing primary amines (e.g., Tris or glycine) during conjugation because they compete with the intended reaction. If necessary, dialyze or desalt samples into a buffer such as phosphate buffered saline (PBS).

• The reagent-to-protein molar ratio in the reaction affects the number of amino groups modified. Optimize this ratio to obtain the level of modification needed for the specific application.

Additional Materials Required

• Water-miscible organic solvent (molecular sieve-treated) such as dimethylsulfoxide (DMSO) or dimethylformamide (DMF) for preparing reagent stock solution
• Small-volume, non-coring syringes for dispensing reagent stock solution while minimizing exposure to the air
• Phosphate-buffered saline (PBS) or other amine-free buffer at pH 7-8 for use as reaction buffer
• Desalting columns or dialysis units for buffer exchange and removal of excess reagent following modification (e.g., Thermo Scientific Zeba Spin Desalting Columns or Slide-A-Lyzer Dialysis Units)

Procedure for pegylating proteins

The amount of pegylation reagent to use for each reaction depends on the amount of modification desired, the amount of the molecule to be labeled, and its concentration. By regulating the molar ratio of MeO-$(PEG)_n$-NHS ester reagent to target molecule, the extent of labeling can be controlled. As a starting point, consider using a 5- to 20-fold molar excess of the MeO-$(PEG)_n$-NHS ester reagent for protein solutions > 2 mg/mL. When labeling more dilute solutions, a greater relative molar excess of the MeO-$(PEG)_n$-NHS ester reagent may be necessary to achieve the same labeling results. Example calculations for a typical antibody modification are provided for convenience.

A. **Prepare a 250mM Reagent Stock Solution**

1. Read the Important Product Information (previous section) before preparing and storing this solution.
2. Remove vial of reagent from -20°C storage and fully equilibrate it to room temperature before opening.
3. Prepare a 250mM MeO-$(PEG)_n$-NHS ester reagent Stock Solution by dissolving 100 mg of reagent in the right volume of dry water-miscible solvent (e.g., dry DMF or DMSO):

B. Calculate the Amount of Reagent Needed

1. Calculate the quantity in millimoles of MeO-$(PEG)_n$-NHS ester reagent to add to the reaction for a 20-fold molar excess:

2. Calculate microliters of 250mM MeO-$(PEG)_n$-NHS ester reagent stock solution (prepared in Step A3) to add to the reaction.

C. Labeling Reaction

1. Dissolve 1-10 mg protein to be modified in PBS according to the calculations made in section A.

Note: Protein that is already dissolved in amine-free buffer at pH 7.2-8.0 may be used without buffer exchange or dilution with PBS. Proteins in Tris or other amine-containing buffers must be exchanged into a suitable buffer.

2. Remove vial of MeO-$(PEG)_n$-NHS ester reagent Stock Solution from storage and fully equilibrate it to room temperature before use.
3. Using a syringe, remove an appropriate volume (see Calculations in section A) of the 250mM MeO-$(PEG)_n$-NHS ester reagent Stock Solution, dispense it into the protein solution and mix well. If the required volume is too small to dispense accurately, remove a portion and prepare a diluted stock solution

by adding additional dry solvent. Add the appropriate volume of diluted Reagent Stock Solution to the protein solution. Discard excess diluted reagent.

4. Incubate reaction on ice for two hours or at room temperature for 30 minutes.

Note: Other than the possibility of ordinary protein degradation or microbial growth, there is no harm in reacting longer than the specified time.

5. Labeling is complete at this point and, although unreacted and hydrolyzed pegylation reagent remains in the solution, it is often possible to perform preliminary tests of the labeled protein. Once proper function and labeling has been confirmed, the labeled protein may be purified from unreacted pegylation reagents and byproduct using desalting or dialysis.

References:

• Hermanson, G.T. (2008). *Bioconjugate Techniques*, Academic Press (Part No. 20036)

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