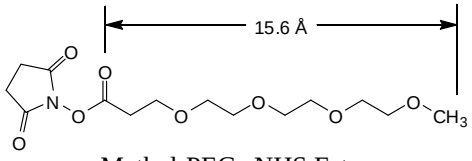
INSTRUCTIONS

$MS(PEG)_n$ Reagents

Branched and unbranched amine-reactive PEGylation reagents

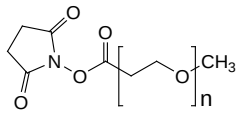
MAN0016373
Rev. A.0
Pub. Part No. 2161767.6

Number	Description
22341	**$MS(PEG)_4$**, 100mg
22342	**$MS(PEG)_4$**, 1g Form: Viscous liquid Molecular Weight: 333.33 Spacer Arm: 15.6Å



Methyl-PEG_4-NHS Ester

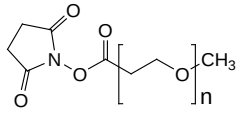
22509	**$MS(PEG)_8$**, 100mg Form: Viscous liquid Molecular Weight: 509.54 Spacer Arm: 30.8Å



n = 8

Methyl-PEG_8-NHS Ester

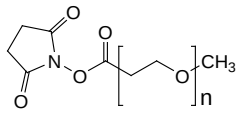
22685	**$MS(PEG)_{12}$**, 100mg
22686	**$MS(PEG)_{12}$**, 1g Form: Viscous liquid Molecular Weight: 685.71 Spacer Arm: 44.9Å



n = 12

Methyl-PEG_{12}-NHS Ester

22687	**$MS(PEG)_{24}$**, 100mg Form: Viscous liquid Molecular Weight: 1214.39 Spacer Arm: 88.2Å

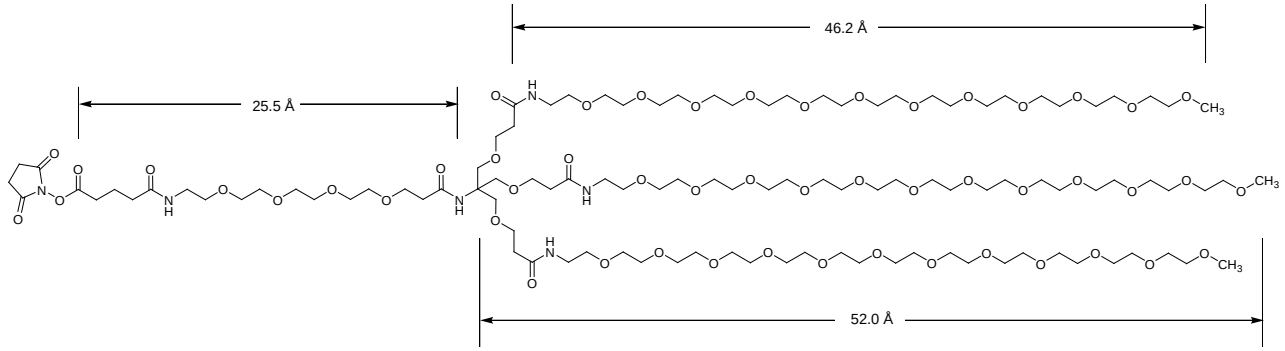


n = 24

Methyl-PEG_{24}-NHS Ester

22421	**$TMS(PEG)_{12}$**, 100mg
22424	**$TMS(PEG)_{12}$**, 1g Form: Waxy, semi-translucent solid Molecular Weight: 2420.80 Spacer Arm: See structure

($Methyl\text{-}PEG_{12})_3\text{-}PEG_4$-NHS Ester



Storage: Upon receipt store desiccated at -20°C. Product is shipped at ambient temperature.

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Introduction

The Thermo Scientific™ MS(PEG)$_n$ and TMS(PEG)$_{12}$ 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, trimethyl succinimidyl (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, Thermo Scientific Pierce 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 (Figure 1). 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 MS(PEG)$_n$ reagents are readily soluble in water or organic solvents such as DMSO, methylene chloride and DMF.

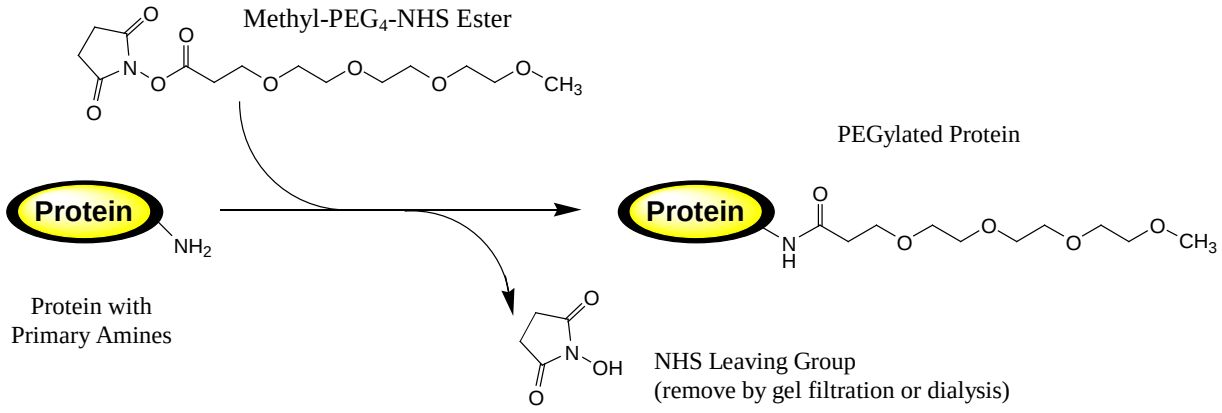

Figure 1. Schematic of protein PEGylation with MS(PEG)$_4$. Proteins are many times larger than the PEGylation reagent and usually contain several amino groups, each of which could be labeled.

Important Product Information

- The MS(PEG)$_n$ reagents are viscous pale liquids that are difficult to weigh and dispense. To facilitate handling, make a stock solution immediately before first use by dissolving the reagent in dry (anhydrous, molecular-sieve treated) organic solvent, such as dimethylformamide (DMF, Product No. 20672) and dimethylsulfoxide (DMSO, Product No. 20688). 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, Product No. 20688) or dimethylformamide (DMF, Product No. 20673) 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 (see Important Product Information and Related Thermo Scientific Products)
- 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 NHS-PEGylation 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 NHS-PEGylation reagent for protein solutions > 2mg/mL. When labeling more dilute solutions, a greater relative molar excess of NHS-PEGylation reagent may be necessary to achieve the same labeling results. Example calculations for a typical antibody modification are provided for convenience.

A. Calculate the Amount of Reagent Needed

1. Calculate the quantity in millimoles of NHS-PEGylation reagent to add to the reaction for a 20-fold molar excess:

$$\text{mL protein} \times \frac{\text{mg protein}}{\text{mL protein}} \times \frac{\text{mmol protein}}{\text{mg protein}} \times \frac{\text{20 mmol PEGylation Reagent}}{\text{mmol protein}} = \text{mmol PEGylation Reagent}$$

Note: the value 20 in this equation corresponds to the suggested reagent molar fold excess for a 2mg/mL protein sample.

2. Calculate microliters of 250mM NHS-PEGylation Reagent stock solution (prepared in Step B.1) to add to the reaction:

$$\text{mmol PEGylation Agent} \times \frac{1{,}000{,}000\ \mu\text{L}}{\text{L}} \times \frac{\text{L}}{250\ \text{mmol}} = \mu\text{L PEGylation Reagent Stock Solution}$$

Example Calculation:

For 1mL of a 2mg/mL IgG (150,000 MW) solution, ~1 µL of 250mM NHS-PEGylation reagent will be added:

$$1\,\text{mL IgG} \times \frac{2\,\text{mg IgG}}{1\,\text{mL IgG}} \times \frac{1\,\text{mmol IgG}}{150{,}000\,\text{mg IgG}} \times \frac{20\,\text{mmol NHS PEGylation Agent}}{1\,\text{mmol IgG}} = 0.000266\,\text{mmol PEGylation Reagent}$$

$$0.000266\,\text{mmol PEGylation Reagent} \times \frac{1{,}000{,}000\,\mu\text{L}}{\text{L}} \times \frac{\text{L}}{250\,\text{mmol}} = 1.07\,\mu\text{L of 250 mM PEGylation Reagent Stock Solution}$$

B. Prepare 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 PEGylation Reagent Stock Solution by dissolving 100mg of reagent (i.e., entire contents of vial, approximately 100µL) in the following volume of dry water-miscible solvent (e.g., dry DMF or DMSO):
   - $MS(PEG)_4$: 1.1mL (For Product No. 22342 make total volume to 12mL)
   - $MS(PEG)_8$: 690µL
   - $MS(PEG)_{12}$: 485µL (For Product No. 22686 make total volume to 5.85mL)
   - $MS(PEG)_{24}$: 230µL
   - $TMS(PEG)_{12}$: 65µL (To make 125mM Stock, add 230µL) (For Product No. 22424 make total volume to 1.65mL)
4. Cap, store and handle stock solutions as directed in the Important Product Information Section.

C. Labeling Reaction

1. Dissolve 1-10mg 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 PEGylation 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 PEGylation 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 excess nonreacted 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 nonreacted PEGylation reagent using desalting or dialysis.

Related Thermo Scientific Products

21330	**EZ-Link™ NHS-PEG$_4$-Biotin,** 25mg
22711	**MM(PEG)$_{12}$,** 100mg (sulfhydryl-reactive Methyl-PEG$_{12}$-Maleimide)
28372	**Phosphate Buffered Saline Packs,** 40 pack, each pack yields 500mL of 0.1M sodium phosphate, 0.15M sodium chloride, pH 7.2 when reconstituted with 500mL water
69576	**Slide-A-Lyzer™ MINI Dialysis Unit Kit,** 10 units plus float
66382, 66807	**Slide-A-Lyzer Dialysis Cassette Kits**
89889	**Zeba Spin Desalting Columns 2mL,** 5 columns
89891	**Zeba Spin Desalting Columns 5mL,** 5 columns

General Reference

Morar, A.S., *et al*. (2006). PEGylation of proteins: A structural approach. *BioPharm. Int.* April 34-46.

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