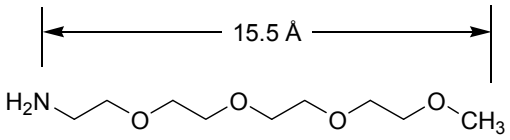
INSTRUCTIONS

MA(PEG)$_n$ Reagents

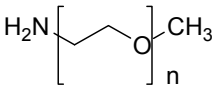
Methyl-amine PEGylation reagents

2074.0

Number	Description
26110	**MA(PEG)$_4$,** 100 mg
26111	**MA(PEG)$_4$,** 1 g Form: Colorless to pale yellow liquid Molecular Weight: 207.27 Spacer Arm: 15.5 Å
26112	**MA(PEG)$_8$,** 100 mg
26113	**MA(PEG)$_8$,** 1 g Form: Colorless to pale yellow liquid at ambient or solid at -20°C Molecular Weight: 383.48 Spacer Arm: 29.7 Å
26114	**MA(PEG)$_{12}$,** 100 mg
26115	**MA(PEG)$_{12}$,** 1 g Form: Colorless to pale yellow liquid at ambient or solid a -20°C Molecular Weight: 559.69 Spacer Arm: 43.9 Å
26116	**MA(PEG)$_{24}$,** 100 mg
26117	**MA(PEG)$_{24}$,** 1 g Form: White to pale yellow waxy solid Molecular Weight: 1088.32 Spacer Arm: 86.1 Å



Methyl-PEG$_4$-Amine



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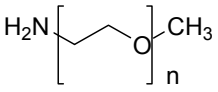
Methyl-PEG$_8$-Amine



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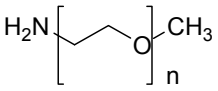
Methyl-PEG$_{12}$-Amine



n = 24
Methyl-PEG$_{24}$-Amine

Storage: Upon receipt store product at -20°C protected from moisture. Product is shipped at ambient temperature.

Introduction

The methyl-PEG$_n$-amine (MA[PEG]$_n$) PEGylation reagents are methyl ether-terminated PEG amines that are used for modifying proteins or surfaces such as beads, nanoparticles and self-assembled monolayers. Modification of proteins adds polyethylene glycol (PEG) spacers, which impart increased water solubility, reduced immunogenicity of the labeled molecule and enhanced *in vivo* stability in solution.[1] Functionalization of solid surfaces, such as quantum dots, self-assembled monolayers and nanoparticles, with polyethylene glycol spacers significantly reduces nonspecific protein binding.[2-7] MA(PEG)$_n$ reagents used with CA(PEG)$_n$ reagents in surface modification can form a hydrophilic "lawn" of methyl ether-terminated PEGs with periodic exposed carboxy-terminated PEGs. The exposed carboxy groups can be coupled to affinity ligands using the carbodiimide coupling reaction with EDC and sulfo-NHS.

Typical PEGylation reagents contain heterogeneous mixtures of different PEG chain lengths; however, our PEGylation reagents are homogenous compounds of defined molecular weight and spacer length, providing precision in optimizing modification applications.

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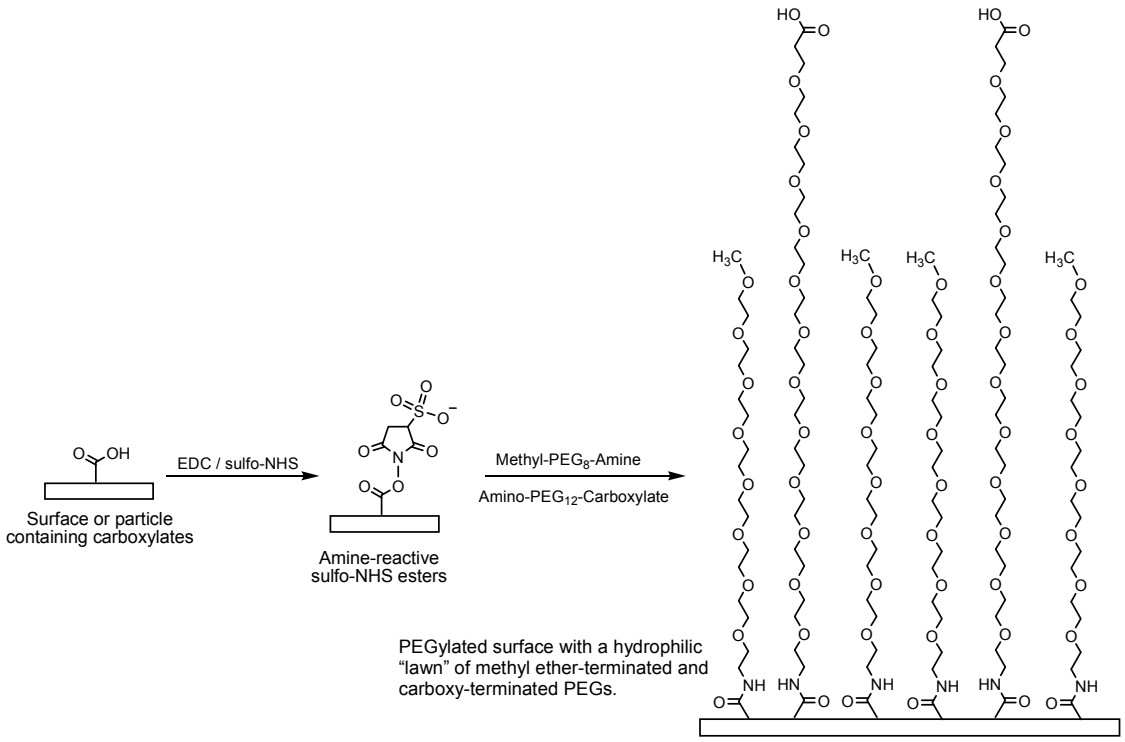

Figure 1. Two-step modification of surfaces with CA(PEG)$_n$–MA(PEG)$_n$ reagents.

Important Product Information

- The MA(PEG)$_n$ reagents are low-melting solids that are difficult to weigh and dispense. To facilitate handling, make a stock solution by dissolving the reagent in dry (anhydrous, molecular-sieve treated) organic solvent, such as dimethylformamide (DMF, Prod. No. 20672) or dimethylsulfoxide (DMSO, Product No. 20684).
- Store unused stock solution in a moisture-free condition (e.g., capped under and 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. To minimize air exposure, cap the stock solution with a septum and use a syringe to remove the solution.
- Use the MA(PEG)$_n$ reagents (see Related Products) in combination with CA(PEG)$_n$ reagents to modify surfaces and minimize nonspecific binding.
- Use non-amine-containing buffers at pH 7-9 such as PBS (20 mM sodium phosphate, 150 mM NaCl; pH 7.4) (Product No. 28372); 20 mM HEPES; 100 mM carbonate/biocarbonate; or 50 mM borate. Do not use buffers that contain primary amines, such as Tris or glycine, which compete with acylation.
- The CA(PEG)$_n$-to-MA(PEG)$_n$ ratio and the reagent mixture-to-surface carboxylic acid molar ratio in the reaction affects the number of carboxy groups modified on the surface and the number of new carboxylic acid residues available for further modification. Optimize these ratios to obtain the modification level needed for the specific application.

Procedure for Coupling CA(PEG)$_n$–MA(PEG)$_n$ Mixtures to Carboxylated Surfaces

The following protocol, adapted from a procedure described by Grabarek and Gergely[8] is a two-step coupling reaction using EDC and NHS or Sulfo-NHS. The CA(PEG)$_n$–MA(PEG)$_n$ mixture is coupled to a carboxylated surface without exposing the carboxylic acids on CA(PEG)$_n$ to EDC. The activation reaction requires quenching with a thiol-containing compound.

The activation reaction with EDC and Sulfo-NHS is most efficient at pH 4.5-7.2; however, the reaction of Sulfo-NHS-activated molecules with primary amines is most efficient at pH 7-8. For best results, perform the first reaction in MES buffer (or other non-amine, non-carboxy buffer) at pH 5-6, then raise the pH to 7.2-7.5 with phosphate buffer (or other non-amine buffer) immediately before reacting with the CA(PEG)$_n$–MA(PEG)$_n$ mixture. Use DTT to quench the activation reaction. The conjugation reaction is quenched using hydroxylamine, Tris or glycine.

Materials Required

- Water-miscible organic solvent (molecular sieve-treated) such as dimethylsulfoxide (DMSO, Product No. 20684) or dimethylformamide (DMF, Product No. 20673) for preparing the reagent stock solution
- Small-volume, non-coring syringes for dispensing the reagent stock solution while minimizing exposure to air
- EDC (Product No. 77149)
- Activation Buffer: MES-buffered saline (0.1 M MES, 0.5 M NaCl; pH 6.0 or 0.1 M MES, 0.9% NaCl; pH 4.7; Product No. 28390)
- Conjugation Buffer: Phosphate-buffered saline, PBS (20 mM sodium phosphate, 0.15 M NaCl; pH 7.2, Product No. 28372)
- NHS or Sulfo-NHS (Product No. 24500 and 24510, respectively)
- Dithiothreitol (DTT; Product No. 20290 or 20291)
- Hydroxylamine•HCl (Product No. 26103)

Procedure

1. Equilibrate EDC, NHS or sulfo-NHS, $CA(PEG)_n$, and $MA(PEG)_n$ to room temperature before opening bottles.
2. Prepare $CA(PEG)_n$ and $MA(PEG)_n$ stock solutions by dissolving 100 mg of each reagent (~100 µl) in the desired amount of dry water-miscible solvent (e.g., DMF or DMSO).
3. Cap, store and handle stock solutions as directed in the Important Product Information Section.
4. Add appropriate amounts of EDC and NHS or sulfo-NHS to the appropriate amount of carboxylated surface in Activation Buffer and react for 15 minutes at room temperature.
5. Add DTT to quench the EDC.

   Note: For surfaces that can be easily washed, the quenching step can be skipped and the surface washed with Coupling Buffer to remove any remaining EDC and NHS.
6. Add the $CA(PEG)_n$–$MA(PEG)_n$ mixture prepared in Conjugation Buffer to the activated surface and react for 2 hours at room temperature.
7. To quench the reaction, add hydroxylamine or another amine-containing buffer. Hydroxylamine hydrolyzes non-reacted NHS on the solid surface and results in hydroxamate formation. Other quenching methods involve adding Tris, lysine, glycine or ethanolamine; however, these primary amine-containing compounds modify carboxyls.

   Note: The newly introduced carboxy groups can be further modified by repeating steps 4 and 5.
8. Add the desired amine-containing substrate, prepared in Coupling Buffer, to the activated surface and react for 2 hours at room temperature.
9. Quench the reaction as described in step 7.

Related Products

26120	**$CA(PEG)_4$,** 100 mg
26121	**$CA(PEG)_4$,** 1 g
26122	**$CA(PEG)_8$,** 100 mg
26123	**$CA(PEG)_8$,** 1 g
26124	**$CA(PEG)_{12}$,** 100 mg
26125	**$CA(PEG)_{12}$,** 1 g
26126	**$CA(PEG)_{24}$,** 100 mg
26127	**$CA(PEG)_{24}$,** 1 g
20684	**DMSO,** 50 ml

20673	**DMF,** 50 ml
28390	**BupH™ MES Buffered Saline,** 10 packs, makes 5 L
28372	**BupH Phosphate Buffered Saline,** 40 packs, makes 20 L
77149	**EDC,** 10 mg
24500	**NHS (*N*-hydroxy succinimide),** 25 g
24510	**Sulfo-NHS (sulfo *N*-hydroxy succinimide),** 500 mg
20290	**DTT,** 5 g
20291	**No-Weigh™ DTT,** 48 tubes × 7.7 mg
26103	**Hydroxylamine,** 25 g

References

1. Morar, A.S., *et al.* (2006). PEGylation of proteins: A structural approach. *BioPharm. Int.* April 34-46.
2. Prime, K.L. and Whitesides, G.M. (1991). Self-assembled organic monolayers: model systems for studying absorption of proteins at surfaces. *Science* **252:**1164.
3. Bentzen, E.L., *et al.* (2005). Surface modification to reduce non-specific binding of quantum dots in live cell assays. *Bioconjugate Chem.* **16:**1488-94.
4. Lin, P-C., *et al.* (2006). Ethylene glycol-protected magnetic nanoparticles for a multiplexed immunoassay in human plasma. *Small* **2(4):**485-9.
5. Zheng, M., *et al.* (2003). Ethylene glycol monolayer protected nanoparticles for eliminating nonspecific binding with biological molecules. *J. Am. Chem. Soc.* **125:**7790-1.
6. Verma, A. and Rotello, V.M. (2005). Surface recognition of biomacromolecules using nanoparticle receptors. *Chem. Commun.* **3:**303-12.
7. Kidambi, S., *et al.* (2004). Selective depositions on polyelectrolyte multilayers: self-assembled monolayers of m-dPEG acid as molecular template. *J. Am. Chem. Soc.* **126:**4697-03.
8. Grabarek, Z. and Gergely, J. (1990). Zero-length crosslinking procedure with the use of active esters. *Anal. Biochem.***185:**131-5.

Current versions of product instructions are available at www.thermo.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.