INSTRUCTIONS

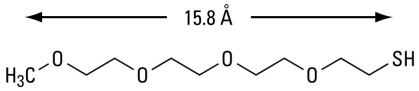
$MT(PEG)_4$
$CT(PEG)_{12}$

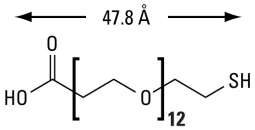
Methyl- and carboxy-thiol PEGylation reagents

26132 26133

2341.0

Number	Description
26132	**$MT(PEG)_4$,** 100mg Molecular Weight: 224.32 Spacer Arm Length: 15.8Å
26133	**$CT(PEG)_{12}$,** 100mg Molecular Weight: 634.77 Spacer Arm Length: 47.8Å





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

Introduction

The Thermo Scientific $MT(PEG)_4$ (methyl-PEG_4-thiol) and $CT(PEG)_{12}$ (carboxy-PEG_{12}-thiol) are monodentate thiol-terminated polyethylene glycol (PEG)-containing reagents with either a methyl ether or carboxylic acid. These reagents have defined molecular weights and spacer lengths and are used for modifying surfaces such quantum dots, self-assembled monolayers and magnetic particles. Functionalization of solid surfaces with polyethylene glycol spacers significantly reduces nonspecific protein binding.[1-6]

The use of $MT(PEG)_4$ with $CT(PEG)_{12}$ in surface modification can form a hydrophilic "lawn" of methyl ether-terminated PEGs with periodic exposed carboxylic acid-containing PEGs. The exposed carboxylic acid groups can be coupled to affinity ligands using the carbodiimide coupling reaction with EDC and sulfo-NHS.

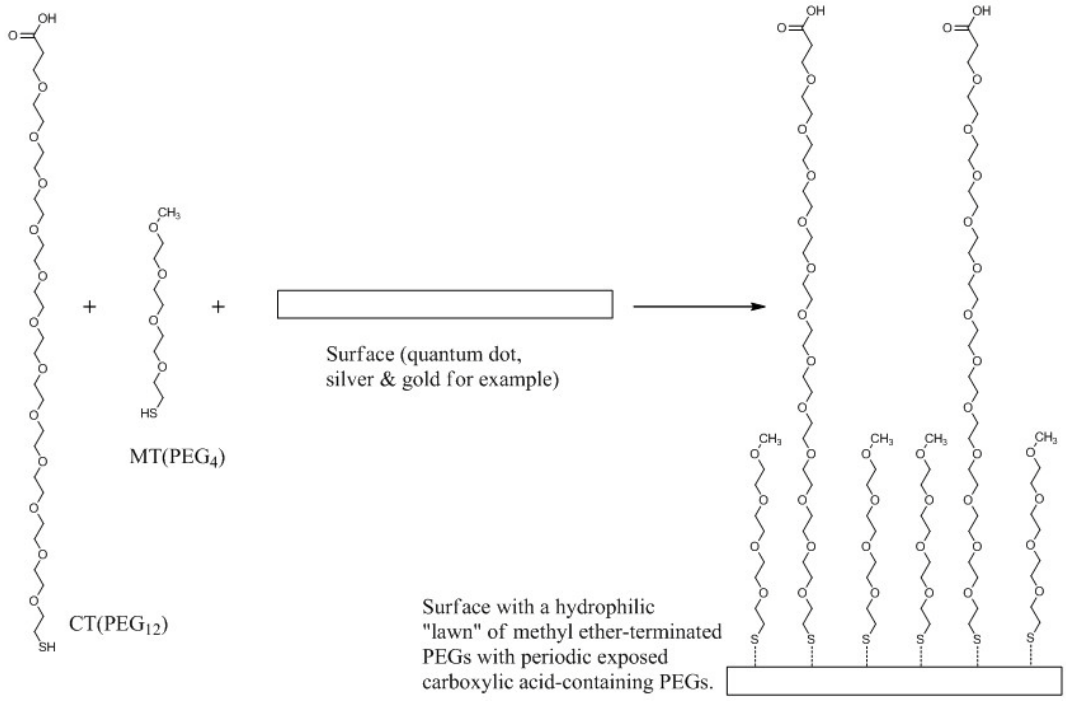


Figure 1. Surface modification with $MT(PEG)_4$ and $CT(PEG)_{12}$.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce

Important Product Information

- Use $MT(PEG)_4$ and $CT(PEG)_{12}$ in combination to modify surfaces and minimize nonspecific binding.
- The PEG-thiol reagents are low-melting solids that are difficult to weigh and dispense. To facilitate handling, make a stock solution by dissolving the reagent in dimethylsulfoxide (DMSO) or dimethylformamide (DMF).
- Store unused stock solution at -20°C. Equilibrate reagent vial to room temperature before opening to avoid moisture condensation. To minimize air exposure, keep the stock solution under an inert gas such as argon or nitrogen. Cap the stock solution with a septum and use a syringe to remove the solution.
- The ratio of $MT(PEG)_4$ to $CT(PEG)_{12}$ and the reagent mixture-to-surface ratio in the reaction affect the number of carboxylic acid residues available for further modification. Optimize these ratios to obtain the modification level needed for the specific application.
- Use non-amine-containing buffers at pH 7-9 such as PBS (20mM sodium phosphate, 150mM NaCl; pH 7.4) (Product No. 28372); 100mM carbonate/biocarbonate; or 50mM borate. Do not use buffers that contain primary amines, such as Tris or glycine, which compete with acylation.

Procedure for Surface Modification with $MT(PEG)_4$ and $CT(PEG)_{12}$

Materials Required

- Water-miscible organic solvent (molecular sieve-treated) such as DMSO or DMF
- Small-volume, non-coring syringes for dispensing the reagent stock solution while minimizing exposure to air
- Buffer A: Phosphate-buffered saline, PBS (20mM sodium phosphate, 0.15M NaCl; pH 7.2, Product No. 28372) or other non-amine, lone-pair sulfur-free buffers
- Buffer B: MES-buffered saline (0.1M MES, 0.5M NaCl; pH 6.0 or 0.1M MES, 0.9% NaCl; pH 4.7; Product No. 28390) or other non-amine, non-carboxy, lone-pair sulfur-free buffers
- EDC (Product No. 77149)
- NHS or Sulfo-NHS (Product No. 24500 and 24510, respectively)
- Hydroxylamine•HCl (Product No. 26103)

Procedure

1. Equilibrate the $MT(PEG)_4$ and $CT(PEG)_{12}$ reagents to room temperature before opening bottles.
2. Prepare stock solutions by dissolving 100mg of each reagent in the desired amount of DMF or DMSO. Cap, store and handle stock solutions as directed in the Important Product Information Section.
3. Prepare the appropriate amount of surface in Buffer A.
4. Prepare a mixture of $MT(PEG)_4$ and $CT(PEG)_{12}$ in Buffer A and add it to the surface. Incubate the reaction for 2 hours at room temperature.
5. Wash the surface with Buffer A to remove excess reagent.
6. The newly introduced carboxylic acid groups can be activated by adding appropriate amounts of EDC and NHS or sulfo-NHS to the modified surface in Buffer B and reacting for 15 minutes at room temperature. For best results, perform this reaction at pH 5-6.

   Note: 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.
7. Wash the surface with Buffer B to remove any remaining EDC and NHS.
8. Add the desired amine-containing substrate, prepared in Buffer A, to the activated surface and react for 2 hours at room temperature. For best results, raise the pH of the reaction solution to 7.2-7.5 with Buffer A immediately before adding the amine-containing substrate.
9. To quench the conjugation reaction, add hydroxylamine or another amine-containing buffer. Hydroxylamine hydrolyzes non-reacted NHS. Other quenching compounds include Tris, lysine, glycine or ethanolamine; however, these primary amine-containing compounds modify carboxylic acids.

Related Thermo Scientific Products

26134	**ML(PEG)$_4$** (methyl-PEG$_4$-lipoamide), 100mg
26135	**CL(PEG)$_{12}$** (carboxy-PEG$_{12}$-lipoamide), 100mg
28390	**BupH™ MES Buffered Saline,** 10 packs, makes 5L
28372	**BupH Phosphate Buffered Saline,** 40 packs, makes 20L
77149	**EDC,** 10mg
24500	**NHS (*N*-hydroxysuccinimide),** 25g
24510	**Sulfo-NHS (sulfo-*N*-hydroxysuccinimide),** 500mg
20290	**DTT,** 5g
20291	**DTT, No-Weigh™ Format,** 48 tubes × 7.7mg
26103	**Hydroxylamine,** 25g
26120	**CA(PEG)$_4$** (carboxy-PEG$_4$-amine), 100mg
26122	**CA(PEG)$_8$,** 100mg
26124	**CA(PEG)$_{12}$,** 100mg
26126	**CA(PEG)$_{24}$,** 100mg
26110	**MA(PEG)$_4$** (methyl-PEG$_4$-amine), 100mg
26112	**MA(PEG)$_8$,** 100mg
26114	**MA(PEG)$_{12}$,** 100mg
26116	**MA(PEG)$_{24}$,** 100mg

References

1. Prime, K.L. and Whitesides, G.M. (1991). Self-assembled organic monolayers: model systems for studying absorption of proteins at surfaces. *Science* **252:**1164.
2. 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.
3. Lin, P-C., *et al.* (2006). Ethylene glycol-protected magnetic nanoparticles for a multiplexed immunoassay in human plasma. *Small* **2(4):**485-9.
4. Zheng, M., *et al.* (2003). Ethylene glycol monolayer protected nanoparticles for eliminating nonspecific binding with biological molecules. *J Am Chem Soc* **125:**7790-1.
5. Verma, A. and Rotello, V.M. (2005). Surface recognition of biomacromolecules using nanoparticle receptors. *Chem Commun* **3:**303-12.
6. 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.

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