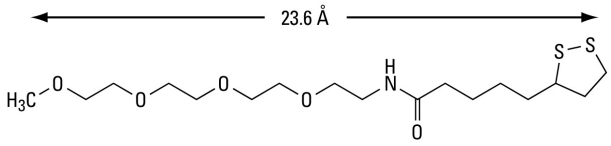
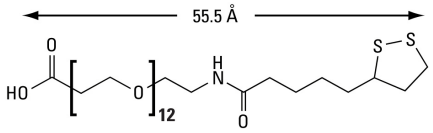
INSTRUCTIONS

ML(PEG)$_4$
CL(PEG)$_{12}$

Methyl- and carboxy-lipoamide PEGylation reagents

26134 26135

2342.0

Number	Description	
26134	**ML(PEG)$_4$,** 100mg Molecular Weight: 395.58 Spacer Arm Length: 23.6Å	
26135	**CL(PEG)$_{12}$,** 100mg Molecular Weight: 806.03 Spacer Arm Length: 55.5Å	

Storage: Upon receipt store at 4°C protected from moisture. Products shipped at ambient temperature.

Introduction

The Thermo Scientific ML(PEG)$_4$ (methyl-PEG$_4$-lipoamide) and CL(PEG)$_{12}$ (carboxy-PEG$_{12}$-lipoamide) are bidentate thiol-terminated polyethylene glycol (PEG)-containing reagents with either a methyl ether or carboxylic acid. These reagents are used for modifying surfaces such as quantum dots, self-assembled monolayers, and magnetic particles. Functionalization of solid surfaces, such as quantum dots and silver and gold surfaces with polyethylene glycol spacers significantly reduces nonspecific protein binding.[1-6]

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

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

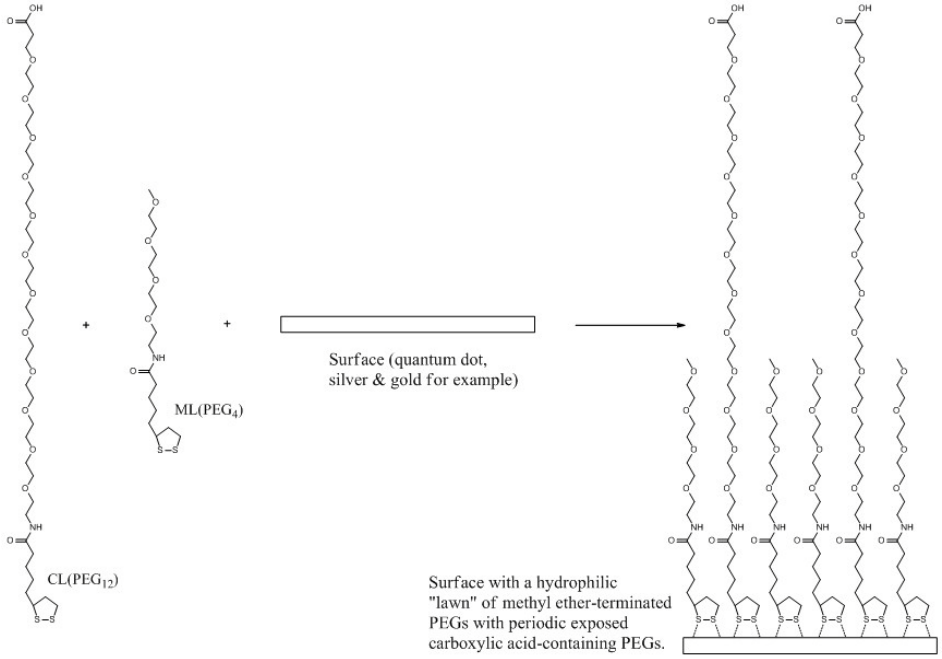


Figure 1. Surface modification with ML(PEG)$_4$ and CL(PEG)$_{12}$.

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Important Product Information

- Use ML(PEG)$_4$ and CL(PEG)$_{12}$ in combination to modify surfaces and minimize nonspecific binding.
- The PEG-lipoamide reagents are low-melting solids that are difficult to weigh and dispense. To facilitate handling, make a stock solution by dissolving the reagent with 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 ML(PEG)$_4$ to CL(PEG)$_{12}$ and the mixture-to-surface ratio affect the number of carboxylic acid residues available for further modification. Optimize these ratios to obtain the ideal modification level for the specific application.
- Use non-amine-containing buffers at pH 7-9 such as phosphate-buffered saline (PBS; 20mM sodium phosphate, 150mM NaCl; pH 7.4; Product No. 28372), carbonate/biocarbonate (100mM) or borate (50mM). Do not use buffers that contain primary amines, such as Tris or glycine, which compete with acylation.

Procedure for coupling PEG-lipoamide reagents to a surface

Materials Required

- Water-miscible organic solvent (molecular sieve-treated) such as DMSO or DMF
- Small-volume, non-coring syringes for dispensing the 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 buffer.
- 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 ML(PEG)$_4$ and CL(PEG)$_{12}$ to room temperature before opening bottles.
2. Prepare stock solutions by dissolving 100mg of each PEG-lipoamide reagent 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. Prepare the surface in Buffer A.
5. Prepare a mixture of ML(PEG)$_4$ and CL(PEG)$_{12}$ in Buffer A and add it to the surface. Incubate the reaction for 2 hours at room temperature.
6. Wash the surface with Buffer A to remove excess reagent.
7. 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.
8. Wash the surface with Buffer B to remove any remaining EDC and NHS.
9. 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.
10. To quench the conjugation reaction, add hydroxylamine or other 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

20688	**Dimethylsulfoxide (DMSO), Sequanal grade,** 950mL
20673	**Dimethylformamide (DMF), Sequanal grade,** 50mL
26132	**MT(PEG)$_4$** (methyl-PEG$_4$-thiol), 100mg
26133	**CT(PEG)$_{12}$** (carboxy-PEG$_{12}$-thiol), 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*-hydroxy succinimide),** 25g
24510	**Sulfo-NHS (sulfo *N*-hydroxy succinimide),** 500mg
20290	**DTT,** 5g
20291	**DTT, No-Weigh™ Format,** 48 tubes × 7.7mg
26103	**Hydroxylamine,** 25g

References

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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.