Instructions for $(PEG)_n$-Thiol Reagents

Introduction

The BroadPharm $(PEG)_n$-thiol is a thiol-terminated polyethylene glycol (PEG)-containing reagent with either a methyl ether, hydroxyl or carboxylic acid group at the other end. 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

Product Information

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

• The $(PEG)_n$-thiol reagents are low-melting solids or liquid 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.

•If the Carboxyl- $(PEG)_n$-thiol reagent is used for surface binding and further protein loading, the reagent-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); 100mM carbonate/biocarbonate; or 50mM borate. Do not use buffers that contain primary amines, such as Tris or glycine, which compete with acylation

Additional 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, 0.15MNaCl; pH 7.2) 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) or other non-amine, non-carboxy, lone-pair sulfur-free buffers

• EDC

• NHS

• Hydroxylamine•HCl

Procedure

1. Equilibrate the $(PEG)_n$-thiol reagent to room temperature before opening bottle.

2. Prepare stock solutions by dissolving 100 mg 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 desired amount of the $(PEG)_n$-thiol reagent 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. If using the Carboxyl- $(PEG)_n$-thiol reagent, the newly introduced carboxylic acid groups can be activated by adding appropriate amounts of EDC and 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 NHS is most efficient at pH 4.5-7.2; however, the reaction of 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.

References

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