Instructions for the use of (PEG)n-Amine Reagents

Introduction

The(PEG)n-amine reagents are used for modifying proteins or surfaces such as beads, nanoparticles and self-assembled monolayers. Modification of proteins adds polyethylene glycol $(PEG)_n$ spacers, which impart increased water solubility, reduced immunogenicity of the labeled molecule and enhanced *in vivo* stability in solution.Functionalization of solid surfaces, such as quantum dots, self-assembled monolayers and nanoparticles, with polyethylene glycol spacers significantly reduces nonspecific protein binding. PEG-amine reagents used 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, BroadPharm's pegylation reagents are homogenous compounds of defined molecular weight and spacer length, providing precision in optimizing modification applications.

Product Information

• The (PEG)n-amine reagents are liquid or 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).

• Use non-amine-containing buffers at pH 7-9 such as PBS (20mM sodium phosphate, 150mM NaCl; pH 7.4); 20mM HEPES; 100mM carbonate/biocarbonate; or 50mM borate. Do not use buffers that contain primary amines, such as Tris or glycine, which compete with acylation.

General procedure for coupling PEG-amine reagents tocarboxylated surfaces

The following protocol, adapted from a procedure described by Grabarek and Gergely8 is a two-step coupling reaction using EDC and NHS or Sulfo-NHS. The **(**$PEG)_n$-amine is coupled to a carboxylated surface. 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 PEG - amine reagent. 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 DMSO or DMF for preparing the reagent stock solution

• Small-volume, non-coring syringes for dispensing the reagent stock solution while minimizing exposure to air

• EDC
• Activation Buffer: MES-buffered saline (0.1M MES, 0.5M NaCl; pH 6.0 or 0.1M MES, 0.9% NaCl; pH 4.7)
• Conjugation Buffer: Phosphate-buffered saline, PBS (20mM sodium phosphate, 0.15M NaCl; pH 7.2)
• NHS or Sulfo-NHS
• Dithiothreitol
• Hydroxylamine•HCl

Procedure

1. Equilibrate EDC, NHS or sulfo-NHS, PEG-amine to room temperature before opening bottles.
2. Prepare (PEG)n-amine 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. 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.
4. 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. Also, if the (PEG)n-amine will be added in step 5 is a non-carboxyl containing compound, there is no need to quench the EDC.

5. Add the (PEG)n-amine (carboxy PEG amine alone or the mixed with methyl-PEG-amine) prepared in Conjugation Buffer to the activated surface and react for 2 hours at room temperature.
6. 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.

7. Add the desired amine-containing substrate, prepared in Coupling Buffer, to the activated surface and react for 2 hours at room temperature.
8. Quench the reaction as described in step 7.

References

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