Instructions for the use of PEG Acid

1. Introduction

PEG acids are a class of PEG compounds that have a carboxylic acid group at one end and a hydroxyl, azide, amino, maleimide, or triple bond at the other end. These reagents have a defined molecular weight and interval length and are used to modify proteins or amine-containing surfaces, such as quantum dots, self-assembled monolayers, and magnetic particles. Functionalization of solid surfaces with PEG spacers can significantly reduce non-specific protein binding.

2. Product information

Storage: Store dry in -20℃

(1) Most PEG Acid reagents are low-melting solids that are difficult to weigh and dispense. For ease of handling, make a stock solution by dissolving the reagent in dimethylsulfoxide (DMSO) or dimethylformamide (DMF).

(2) Store unused stock solutions at -20 ° C. Allow the reagent bottle to stand at 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. Cover the stock solution with a septum, and then remove the solution with a syringe.

(3) If PEG acid is used for surface binding and further loading of proteins, the ratio of reagent to surface in the reaction affects the number of carboxylic acid residues available for further modification.

(4) 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.

3. Additional Materials Required

- Water-soluble organic solvents (molecular sieve treatment), such as DMSO or DMF.
- Use a small volume coreless syringe for dispensing reagent stocks while minimizing exposure to air.
- Buffer A: Phosphate buffer, PBS (20 mM sodium phosphate, 0.15 M NaCl; pH 7.2) or other non-amine, single pair of sulfur-free buffer
- Buffer B: MES buffered saline (0.1M MES, 0.5 M NaCl; pH 6.0 or 0.1M MES, 0.9% NaCl; pH 4.7) or other non-amine, non-carboxyl, single pair sulfur-free buffer
- EDC
- NHS
- Hydroxylamine•HCl

4. Procedure

1. Before opening the bottle, let the PEG acid reagent stand at room temperature.

2. Dissolve 100 mg of each reagent in the required amount of DMF or DMSO to prepare a stock solution.
3. Prepare an appropriate amount of surface or protein in buffer A.
4. The carboxylic acid group on the PEG linker can be activated by adding appropriate amounts of EDC and NHS to the modified surface in a small amount of buffer B and reacting at room temperature for 15 minutes. For best results, perform this reaction at pH 5-6.
Note: The activation reaction with EDC and NHS is most effective at pH 4.5-7.2. However, the reaction of NHS-activated molecules with primary amines is most effective at pH 7-8.
5. Add the desired amine-containing substrate prepared in buffer A to the activated surface and react it for 2 hours at room temperature. For best results, increase the pH of the reaction solution to 7.2-7.5 buffer A and add the amine-containing substrate immediately.
6. To quench the conjugation reaction, add hydroxylamine or other amine-containing buffers. Hydroxylamine hydrolyzes unreacted NHS. Other quenching compounds include Tris, lysine, glycine or ethanolamine; however, these major amine-containing compounds can modify carboxylic acids.

5. References

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5. Verma, A. and Rotello, V.M. (**2005**). Surface recognition of biomacromolecules using nanoparticle receptors.ChemCommun3:303-12.
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