

Instructions for NHS-(PEG)_n-Bromide

Introduction

BroadPharm NHS-(PEG)_n-Bromide is a sulfhydryl-reactive and amine-reactive heterobifunctional crosslinker. The reagent's NHS ester reacts with primary amines at pH 7-9 to form stable amide bonds, and the bromoacetyl reacts with sulfhydryl groups at pH >7.5 to form stable thioether bonds. This reagent is useful for preparing cyclic peptides and peptide conjugates because the spacer maintains peptide-like character in the crosslinked species.

Product Information

- Storage : Upon receipt store desiccated at -20°C.
- NHS-(PEG)_n-Bromide is moisture-sensitive. To avoid moisture condensation onto the product, vial must be equilibrated to room temperature before opening.
- Prepare NHS-(PEG)_n-Bromide immediately before use. The NHS-ester moiety readily hydrolyzes and becomes non-reactive; therefore, do not prepare stock solutions for storage. Discard any unused reconstituted crosslinker.
- Use a non-amine-containing reaction buffer at pH 7-9 such as 20mM sodium phosphate, 0.15M sodium chloride; 20mM HEPES; 100mM carbonate/bicarbonate; or 50mM borate. Avoid using Tris or glycine as buffer components, because they will compete with the intended reaction.
- Exclude reducing agents, such as 2-mercaptoethanol, dithiothreitol, and mercaptoethylamine from reaction buffers, as these compounds will quench the bromoacetyl reactivity.
- Sulfhydryls can be introduced via amine modification using *N*-succinimidyl-S-acetylthioacetate (SATA) or 2-iminothiolane•HCl (Traut's Reagent).

Protocol for Preparing IgG/β-Galactosidase Conjugates

The following protocol is a two-step method in which bromoacetyl-activated IgG is prepared in the first step. The activated IgG is then reacted with free sulfhydryls present on the surface of native β-galactosidase. Modify this method as needed to optimize the ratio of IgG to β-galactosidase.

•Additional Materials Required

- Borate buffer: 50mM sodium borate, pH 8.5, 5mM EDTA
- 1mg/mL IgG in borate buffer (pH 8.5)
- Thermo Scientific Zeba Spin Desalting Columns, 10mL (Thermo Scientific Product No. 89894), or device to remove unreacted reagents
- Cysteine•HCl

B. Method

1. Just before use, dissolve 2 mg of NHS-(PEG)_n-Bromide in 1mL DMSO. Protect solution from light.
2. Add 10 μ L of crosslinker solution to 1 mL of IgG and react for 30 minutes at room temperature.
3. Remove unreacted crosslinker using a desalting column equilibrated with borate buffer (pH 8.5).
4. Add 4 mg of β -galactosidase to the desalted IgG and react for 1 hour at room temperature in the dark.
5. To quench the reaction, add a final concentration of 5mM cysteine and react for 15 minutes at room temperature in the dark.
6. Remove unreacted reagents by desalting or dialysis.