Instruction for PFP-(PEG)$_n$-Bromide

Introduction

BroadPpharrm PFP-(PEG)$_n$-Bromide is a sulfhydryl-reactive and amine-reactive heterobifunctionalcrosslinker. The reagent's PFP ester reacts with primary amines at pH 7-9 to form stable amide bonds, and the bromacetyl 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.

Important Product Information

• PFP-(PEG)$_n$-Bromideis moisture-sensitive. Store the vial of linker reagent at -20°C with desiccant. To avoid moisture condensation onto the product, equilibrate vial to room temperature before opening.

• As directed in the procedure, dissolve the PEG reagent immediately before use. The PFP moiety hydrolyzes and becomes non-reactive; therefore, weigh and dissolve only a small amount of the reagent at a time, and do not prepare stock solutions for storage. Discard any unused reconstituted reagent.

• Avoid buffers containing primary amines (e.g., Tris or glycine) as these will compete with the reaction. If necessary, dialyze or desalt to exchange the protein sample into an amine-free buffer such as phosphate-buffered saline (PBS).

• When pegylating the protein, unreacted linker is easily removed by size exclusion using either desalting columns or dialysis. A 10 mL desalting column is best suited for processing pegylation reactions involving 1-10 mg of protein in approximately 0.5-2 mL.

• Exclude reducing agents, such as 2-mercaptoethanol, dithiothreitol, and mercaptoethylamine from reaction buffers, as these compounds will quench the bromacetyl 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 bromacetyl-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.

A. Additional Materials Required

• Borate buffer: 50mM sodium borate, pH 8.5, 5mM EDTA

• 1mg/mL IgG in borate buffer

• Thermo Scientific Zeba Spin Desalting Columns, 10 mL (Thermo Scientific Product No. 89894), or device to remove unreacted reagents

• Cysteine•HCl

B. Method

1. Just before use, dissolve 2 mg of PFP-$(PEG)_n$-Bromide in 1 mL DMSO. Protect solution from light.
2. Add 10 µL of the 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.
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.