Instructions for the use of PFP-(PEG)_n-Azido

Introduction

The BroadPharm PFP-(PEG)_n-Azido is a Azido-containing PEG-PFP active ester which can react with primary and secondary amines. The pentafluorophenyl (PFP) ester-activated PEG linker is less subject to hydrolysis than NHS esters. PFP-(PEG)_n-Azido must be first dissolved in a minimal amount of an organic solvent, such as dimethyl sulfoxide (DMSO) or dimethylformamide (DMF) and then added to the buffer containing the protein or other molecule. The reagent forms an emulsion that allows the reaction to proceed.

Product Information

- PFP-(PEG)_n-Azido is moisture-sensitive. Store the vial of 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).

In the protein pegylation process, 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. For smaller amounts of protein and/or smaller reaction volumes, both the azidolation reaction and subsequent buffer exchange may be performed in a single Thermo Scientific Slide-A-Lyzer MINI Dialysis Unit.

Additional Materials Required

- Phosphate-buffered Saline (PBS): 0.1M phosphate, 0.15M sodium chloride; pH 7.2 or other non-amine containing buffer at pH 7.0-8.0
- Quenching Buffer: Tris-buffered saline (TBS; 25mM Tris, 0.15M sodium chloride; pH 7.2; glycine or other amine-containing buffer)
- Water-miscible organic solvent such as dimethyl sulfoxide (DMSO) or dimethylformamide (DMF)
- 10-100 μL sample volumes; Slide-A-Lyzer® Dialysis Cassette Kit for 0.1-30.0 mL sample volumes; or Zeba Spin Desalting Columns for sample volumes ranging from >10 μL to 4 mL

General Procedure for pegylation of IgG and other Proteins

The degree of (PEG)_n-Azido incorporation can vary depending on the parameters of the pegylation reaction, including protein concentration, PFP-(PEG)_n-Azido concentration, pH and time. Commonly
used reaction conditions include incubation at 4-37°C, pH values from 7 to 9, and incubation times from a few minutes to overnight.

1. Dissolve 2 mg of IgG in 1 mL of PBS (for example, 0.1M sodium phosphate 0.15M NaCl, pH 7.2).
2. Immediately before use, dissolve 1 mg of PFP-(PEG)n-Azido in 75 μL of DMF or DMSO. Add 25 μL of the PFP-(PEG)n-Azido solution to the IgG solution.
3. Incubate the reaction on ice for two hours at room temperature or 37°C for 30 minutes.
4. Remove the unreacted PFP-(PEG)n-Azido by dialysis or gel filtration.
5. Store the pegylated protein at the specified conditions for the unpegylated protein.

Applications:

Two very active areas that use the azide functionality are a) “Click” chemistry, the particular example of the Cu(I) catalyzed reaction of the azide and a terminal acetylene; and b) the Staudinger ligation using functionalized diarylphosphines to couple the azide in a covalent fashion to form amides. For particular “Click” chemistry protocols, please look in the references cited or more detailed application references contained within.
