General method for PFP-$(PEG)_n$ conjugation

Introduction

The BroadPharm PFP-$(PEG)_n$ are PEG labeling reagents that react with primary and secondary amines. The pentafluorophenyl (PFP) ester-activated PEG linker is less subject to hydrolysis than NHS esters, resulting in more efficient reactions. PFP-$(PEG)_n$must first be dissolved in a minimal amount of an organic solvent, such as dimethylsulfoxide (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-$(PEG)_n$ 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 PFP-$(PEG)_n$ reagent immediately before use. The PFP moiety readily 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).

• During the 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 pegylation 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 dimethylsulfoxide (DMSO) or dimethylformamide (DMF)

• 10-100 µL sample volumes; Slide-A-Lyzer® Dialysis Cassette Kit for 0.1-30.0mL sample volumes; or Zeba Spin Desalting Columns for sample volumes ranging from >10 µL to 4 mL

General Procedure for the pegylationof IgG and other Proteins

The following protocol typically results in approximately two to five PEG molecules per IgG. The degree of PEG linker incorporation can vary depending on the parameters of the pegylation reaction, including protein concentration, PFP-$(PEG)_n$ 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-$(PEG)_n$ (in 75 μL of DMF or DMSO. Add 25 μL of the PFP-PEG 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 unreacted PFP-$(PEG)_n$ by dialysis or gel filtration.

5. Store the pegylated protein at at the same conditions specified for the unpegylated protein, until ready for use.