PEG PFP Ester Protocol

1. Introduction

Polyethylene glycol PFP esters are a type of polyethylene glycol labeling reagent that reacts with primary and secondary amines. Compared with NHS ester, PFP ester is not easy to hydrolyze and has higher reaction efficiency. Polyethylene glycol PFP ester must first be dissolved in a small amount of organic solvents, such as dimethyl sulfoxide (DMSO) or dimethylformamide (DMF), and then added to a buffer containing proteins or other molecules. The reagent forms an emulsion, allowing the reaction to proceed.

2. Product Information

(1) PEG PFP Ester is moisture-sensitive. Store the reagent in a desiccant at -20 ° C. To prevent moisture from condensing on the product, equilibrate the vial to room temperature before opening.

(2) As directed in the procedure, dissolve the PEG PFP Ester 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.

(3) Avoid using primary amine-containing buffers (such as Tris or glycine), as they will compete with the reaction. If necessary, perform dialysis or desalting to exchange protein samples for amine-free buffers, such as phosphate buffered saline (PBS).

(4) During the PEGylation process, unreacted linker is easily removed by size exclusion using either desalting columns or dialysis. A 10mL desalting column is best suited for processing PEGylation reactions involving 1-10mg of protein in approximately 0.5-2mL. 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.

3. Additional Materials Required

- Phosphate buffered saline (PBS): 0.1M phosphate, 0.15M sodium chloride; pH 7.2 or other pH 7.0-8.0 buffer solution without amine.
- Quenching buffer: Tris buffered saline (TBS; 25mM Tris, 0.15M sodium chloride; pH 7.2; glycine or other amine-containing buffer).
- Miscible organic solvents, such as dimethyl sulfoxide (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 >10µL to 4mL.

4. General Procedure for the PEGylation of IgG and other Proteins

The following protocols typically result in approximately 2 to 5 PEG molecules per IgG. The degree of PEG linker binding can vary depending on the parameters of the PEGylation reaction, including protein concentration, PEG-PFP ester concentration, pH, and time. Common reaction conditions include incubation at 4-37 ° C, pH values from 7 to 9, and incubation times from minutes to overnight.

(1) Dissolve 2mg of IgG in 1mL of PBS (for example, 0.1M sodium phosphate, 0.15M NaCl, pH 7.2).

(2) Immediately before use, dissolve 1 mg of PEG PFP ester (dissolved in 75μL DMF or DMSO. Add 25μL of PFPPEG solution to the IgG solution.

(3) Incubate the reaction on ice at room temperature or 37 ° C for 2 hours and 30 minutes.

(4) Remove unreacted PEG-PFP ester by dialysis or gel filtration.

(5) Store PEGylated proteins under the same conditions as those specified for non-PEGylated proteins until use.