

## General method for PEG PFP ester conjugation

### Introduction

The PEG PFP ester is a class of PEG labeling reagents that react with primary and secondary amines. The pentafluorophenyl (PFP) ester is less subject to hydrolysis than NHS esters, resulting in more efficient reactions. PEG PFP ester 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

- PEG PFP ester 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 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.
- 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 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.

### 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  $\mu$ L sample volumes; Slide-A-Lyzer<sup>®</sup> Dialysis Cassette Kit for 0.1-30.0mL sample volumes; or Zeba Spin Desalting Columns for sample volumes ranging from >10 $\mu$ L to 4mL

### General Procedure for the PEGylation of 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, PEG PFP ester 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 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 (in 75 $\mu$ L of DMF or DMSO. Add 25 $\mu$ L 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 PEG PFP ester by dialysis or gel filtration.
5. Store the PEGylated protein at the same conditions specified for the non-PEGylated protein, until ready for use.