

## Instructions for PEG NHS Ester Conjugation

### I. Introduction

PEG NHS Ester is a class of amine-reactive reagents with selected length of PEG spacer. This reagent is soluble in organic solvents such as DMSO or DMF. Once dissolved in an organic solvent, the reagent is further diluted in a non-amine containing aqueous buffer. PEG NHS Ester react efficiently with primary amino groups ( $-NH_2$ ) in neutral or slightly basic buffers to form stable amide bonds. Because antibodies and other proteins generally contain multiple lysine (K) residues in addition to the N-terminus of each polypeptide, they have multiple primary amines available as targets for labeling with NHS-activated PEG reagents.

### II. Product Information

- The PEG NHS Ester is moisture-sensitive. Store the vial of the reagent at  $-20^{\circ}C$  with desiccant. To avoid moisture condensation onto the product, equilibrate vial to room temperature before opening.
- Dissolve the PEG NHS Ester immediately before use. The NHS-ester 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 compete with the intended reaction. If necessary, dialyze or otherwise desalt to exchange the protein sample into an amine-free buffer such as phosphate buffered saline.

### III. 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 buffers
- 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

#### IV. Procedure for labeling IgG with PEG NHS Ester

##### A. Calculations

The extent of PEG linker labeling depends on the size and distribution of amino groups on the protein and the amount of reagent used. Compared to reactions involving concentrated protein solutions, labeling reactions with dilute protein solutions require a greater fold molar excess of the PEG NHS Ester linker to achieve the same incorporation level. Typically using a 20-fold molar excess of the PEG NHS Ester linker to label 1-10mg/mL antibody (IgG) results in 4-6 linkers labeling per antibody molecule. Adjust the molar ratio of NHS-(PEG)<sub>n</sub> to protein to obtain the desired level of incorporation.

1. Calculate millimoles of PEG NHS Ester to add to the reaction for a 20-fold molar excess.
2. Calculate microliters of 10mM PEG NHS Ester preparation for adding to the reaction.

##### B. PEG NHS Ester Labeling Reaction

For reaction volumes from 10 $\mu$ L to 100 $\mu$ L, the buffer exchange and PEGylation may be conveniently performed in a single Slide-A-Lyzer MINI Dialysis Unit. For reaction volumes from 0.1mL to 30mL, Slide-A-Lyzer Dialysis Cassettes may be used. Alternatively, Zeba Spin Desalting Columns can be used for a faster buffer exchange.

1. Equilibrate the vial of PEG NHS Ester to room temperature before opening in Step 3.
2. Dissolve 1-10mg protein in 0.5-2mL of PBS according to the calculation made.
3. Immediately before use, prepare a 10mM solution of PEG NHS Ester by adding about 5mg into 1mL of DMSO or DMF.
4. Add the appropriate volume of the PEG NHS Ester solution (a 20-fold molar excess) to the protein solution, making sure that the volume of organic solvent does not exceed 10% of the final reaction volume.
5. Incubate reaction on ice for two hours or at room temperature for 30-60 minutes.
6. Remove the unreacted PEG NHS Ester by dialysis or gel filtration.
7. Store the PEGylated protein using the same condition that is optimal for the non-PEGylated protein.

#### V. Procedure for amine bearing small molecular modification with PEG NHS Ester

1. Slowly dissolve amine bearing small molecules in organic solvents such as DMF, CH<sub>2</sub>Cl<sub>2</sub>, DMSO, THF, or other solvents as needed.
2. Under continuous stirring, PEG NHS Ester was added to the above reaction mixture 1:1 or 2:1 equivalent by mmol depending on the reaction kinetics.
3. The reaction mixture was stirred for 3-24 hours depending on the substrates properties, monitored either by LC-MS or TLC plate.
4. The final product can be isolated by general organic synthesis workup or by column purification.