

PEG NHS Ester Protocol

1. Introduction

PEG-NHS esters are a class of amine-reactive reagents with a certain length of PEG spacer. This reagent is soluble in organic solvents such as DMSO or DMF. Once dissolved in an organic solvent, the reagents are further diluted in an amine-free water buffer. PEG-NHS esters react effectively with primary amino groups (-NH₂) in neutral or weakly basic buffers to form stable amide bonds. Because antibodies and other proteins often contain multiple lysine (K) residues in addition to the N-terminus of each polypeptide, they have multiple primary amines that can be used as targets for NHS-activated PEG reagent labeling.

2. Product information

- PEG-NHS ester is sensitive to moisture. Store the reagent bottle with desiccant at -20 ° C. To prevent moisture from condensing on the product, equilibrate the vial to room temperature before opening.
- Dissolve PEG-NHS ester immediately before use. The urethane part is easily hydrolyzed and does not react; therefore, only a small amount of reagent is weighed and dissolved at one time. Do not prepare a storage solution. Discard unused recombinant reagents.
- Avoid buffers containing primary amines (such as Tris or glycine) as they will compete with the expected reaction. If necessary, perform dialysis or desalting to exchange protein samples for amine-free buffers, such as phosphate buffers.

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 (trimethylolaminomethane) buffered saline (TBS): 25mM Tris, 0.15M sodium chloride; pH 7.2; glycine or other amine-containing buffer.
- Water-soluble organic solvents, such as dimethyl sulfoxide (DMSO) or dimethylformamide (DMF)
- (4) 10-100 microliter sample volume; Slide-A-Lyzer® dialysis cartridge kit with 0.1-30.0ml sample volume; or Zeba rotary desalting column larger than 10 microliter to 4ml sample volume.

4. Procedure for labeling IgG with PEG NHS Ester

A. Calculation

The degree of PEG linker labeling depends on the size and distribution of amino groups on the protein and the number of reagents used. Compared to reactions involving concentrated protein solutions, labeling reactions with dilute protein

solutions requires more molar amounts than PEG NHS ester linker to reach the same level of incorporation. Usually 20-fold PEG-NHS ester linker is used to label 1-10 mg / mL antibody (IgG). As a result, 4-6 linkers are labeled per antibody molecule. Adjust the molar ratio of NHS- (PEG) n to protein to obtain the ideal incorporation level

- (1) Calculate the number of micromoles of polyethylene glycol NHS ester and add to the reaction to obtain a molar amount of 20 times.
- (2) Calculate the number of microliters of 10 mM PEG NHS ester preparation added to the reaction.

B. PEG NHS ester labeling reaction

For reaction volumes from 10 μ L to 100 μ L, buffer exchange and PEGylation can be conveniently performed in a single Slide-A-Lyzer MINI dialysis unit. For reaction volumes of 0.1 mL to 30 mL, a Slide-A-Lyzer dialysis cassette can be used. In addition, Zeba spin desalting columns can be used for faster buffer exchange.

- (1) Equilibrate the vial of PEG NHS ester to room temperature before opening in step 3
- (2) According to calculation, 1-10 mg of protein is dissolved in 0.5-2 mL of PBS.
- (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 the reaction on ice for 2 hours or 30-60 minutes at room temperature.
- (6) Remove unreacted PEG NHS ester by dialysis or gel filtration.
- (7) Store PEGylated proteins under the same optimal conditions as non-PEGylated proteins.

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

- (1) Slowly dissolve small molecules containing amines in organic solvents (such as DMF, CH₂Cl₂, DMSO, THF or other required solvents).
- (2) (2). Under continuous stirring, according to the reaction kinetics, PEG NHS ester is added to the above reaction mixture in a molar ratio of 1: 1 or 2: 1 equivalent.
- (3) (3). Depending on the nature of the substrate, stir the reaction mixture for 3-24 hours and monitor by LC-MS or TLC plate.
- (4) (4). The final product can be separated by conventional organic synthesis or column purification.