Instructions for the use of Azido-(PEG)$_n$-NHS

Introduction

The BroadPharm PEG-Linker NHS-(PEG)$_n$-Azido is a triple bond-reactive reagent with an extended spacer arm. 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. The *N*-Hydroxysuccinimide (NHS) ester-activated PEG linker is an amine-reactive reagent. NHS esters react efficiently with primary amino groups (-NH_2) in pH 7-9 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.

Product Information

• The NHS-(PEG)$_n$ linker is moisture-sensitive. Store the vial of the reagent at -20°C with desiccant. To avoid moisture condensation into the product, equilibrate vial to room temperature before opening.

• Dissolve the NHS-(PEG)$_n$ linker 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.

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 mL sample volumes; or Zeba Spin Desalting Columns for sample volumes ranging from >10 µL to 4 mL

Procedure for labeling IgG with NHS-(PEG)$_n$-Azido

A. Calculations

The extent of azido 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 NHS-(PEG)$_n$-Azidolinker reagent to achieve the same incorporation level. Typically using a 20-fold molar excess of NHS-(PEG)$_n$-

Azido reagent to label 1-10 mg/mL antibody (IgG) results in 4-6 PEG linkers per antibody molecule. Adjust the molar ratio of NHS-$(PEG)_n$-Azido to the protein to obtain the desired level of incorporation.

1. Calculate millimoles of NHS-$(PEG)_n$-Azido to add to the reaction for a 20-fold molar excess.

2. Calculate microliters of 10mM NHS-$(PEG)_n$-Azido preparation for adding to the reaction.

B. NHS-$(PEG)_n$-Azido Labeling Reaction

For reaction volumes from 10 µL to 100 µL, the buffer exchange and labeling reaction 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 NHS-$(PEG)_n$-Azido to room temperature before opening in Step 3.
2. Dissolve 1-10 mg protein in 0.5-2 mL of PBS according to the previous calculation made.
3. Immediately before use, prepare a 10mM solution of NHS-$(PEG)_n$-Azido by adding about 5 mg to 1 mL of DMSO or DMF.
4. Add the appropriate volume of the NHS-$(PEG)_n$-Azido 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 NHS-$(PEG)_n$-Azido by dialysis or gel filtration. See instructions provided with the preferred buffer exchange product.
7. Store the labeling protein using the same condition that is optimal for the non-labeled protein.

Applications:

Two very active areas that use the azide functionality are a) “Click” chemistry, the particular example of the Cu(I) catalyzed reaction of the azide and a terminal acetylene; and b) the Staudinger ligation using functionalized diarylphosphines to couple the azide in a covalent fashion to form amides. For particular “Click” chemistry protocols, please look in the references cited or more detailed application references contained within.

Click Applications: a. “Click Chemistry: Diverse Chemical Function form a Few Good Reactions,” H. C. Kolb, M.G. Finn, and K. Barry Sharpless, Angew. Chem., Int. Eng. Ed., 40, 2004-2021 (2001); b. “The growing impact of click chemistry on drug discovery,” H. C. Kolb and K. Barry Sharpless, Drug Discovery Today, 8(24), 128-1137 (2003); c. “Cu(I)-Catalyzed Alkyne-Azide “Click” Cycloadditions from a Mechanistic and Synthetic Perspective,” V. C. Bock, H. Hiemstra and J. H. van Maarseveen, Eur. J. Org. Chem., 51-68 (2006); d. “A3-Type Star Polymers via Click Chemistry,” O. Altintas, B. Yankul, G Hizal and U. Tunca, J. Poly. Sci.: Part A, Polymer Chem., 44, 6458-6465 (2006); e. “Preparation of alumina supported coppernanoparticles and their application in the synthis of 1, 2, 3-triazoles,” M. L. Kantam, et al., J. Mol. Catal. A: Chem., 256, 273-277 (2006); f. “A Rapid and Versatile Method to Label Receptor Ligands Using “Click” Chemistry: Validation with the Muscarinic M1 Antagonist Pirenzepine,” Bioconjugate Chemistry, 17, 1618-1623 (2006).

For Staudinger ligations: a. “The Staudinger Ligation-A Gift to Chemical Biology,” M. Kohn and R. Breinbauer, Angew. Chem. Int. Ed., 43, 3106 (2004); b. “Traceless Staudinger Ligation of GlycosylAzides with TriarylPhosphines: Stereoselective Synthesis of Glycosyl Amides,” A. Bianchi and A. Bernardi, J. Org. Chem., 71, 4565-4577 (2006); c. “Reaction Mechanism and Kinetics of the Traceless Staudinger Ligation,” M. Soelner, B. L. Nilsson and R. T. Raines, J. Amer. Chem. Soc., 128 (27), 8820-8828 (2006). The first reference is an excellent and recentreview in this very active area. Searching for “Staudinger ligation” can yield more and other excellent references.