Instructions for the use of Alkyne-$(PEG)_n$-acid and Alkyne-$(PEG)_n$-NHS

Introduction

The BroadPharm Alkyne-$(PEG)_n$-acid and Alkyne-$(PEG)_n$-NHS ester are pegylation reagents that reacts with primary and secondary amines at the other end. The terminal alkyne group reacts with an azide to produce a stable triazole, also referred to as the Cu(I)catalyzed click reaction. This reaction possesses extreme selectivity and biocompatibility, such that the complimentary reagents can form covalent bonds within richly functionalized biological systems, in some cases, living organisms. The Alkyne-$(PEG)_n$-acid and Alkyne-$(PEG)_n$-NHS esters 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

•The Alkyne-$(PEG)_n$-acid and the Alkyne-$(PEG)_n$-NHS esters are moisture-sensitive. Store thereagent 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 Alkyne-PEG-NHS ester reagent immediately before use. The NHS 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).

• When pegylating proteins in solution, unreacted PEG linker is easily removed by size exclusion using either desalting columns or dialysis. A 10 mL desalting column is best suited for processing pegylation reactions involving 1-10 mg of protein in approximately 0.5-2 mL. 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

• Water-miscible organic solvent (molecular sieve-treated) such as DMSO or DMF

• Small-volume, non-coring syringes for dispensing the reagent stock solution while minimizing exposure to air

• Buffer A: Phosphate-buffered saline, PBS (20mM sodium phosphate, 0.15M NaCl; pH 7.2) or other non-amine, lone-pair sulfur-free buffers

• Buffer B: MES-buffered saline (0.1M MES, 0.5M NaCl; pH 6.0 or 0.1M MES, 0.9% NaCl; pH 4.7) or other non-amine, non-carboxy, lone-pair sulfur-free buffers

• EDC

• NHS
• Hydroxylamine•HCl

A . General Procedure for Alkyne-$(PEG)_n$-acid**/protein Conjugation**

1. Equilibrate the Alkyne-$(PEG)_n$-acid reagents to room temperature before opening bottles.
2. Prepare stock solutions by dissolving 100 mg of each reagent in the desired amount of DMF or DMSO. Cap, store and handle stock solutions as directed in the Important Product Information Section.
3. Prepare the appropriate amount of surface or Protein Buffer A.
4. The carboxylic acid groups on the PEG linker can be activated by adding appropriate amounts of EDC and NHS to the modified surface in small amount of Buffer B and reacting for 15 minutes at room temperature. For best results, perform this reaction at pH 5-6.

Note: The activation reaction with EDC and NHS is most efficient at pH 4.5-7.2; however, the reaction of NHS-activated molecules with primary amines is most efficient at pH 7-8.

5. Add the desired amine-containing substrate, prepared in Buffer A, to the activated surface and react for 2 hours at room temperature. For best results, raise the pH of the reaction solution to 7.2-7.5 with Buffer A immediately before adding the amine-containing substrate.
6. To quench the conjugation reaction, add hydroxylamine or another amine-containing buffer. Hydroxylamine hydrolyzes non-reacted NHS. Other quenching compounds include Tris, lysine, glycine or ethanolamine; however, these primary amine-containing compounds modify carboxylic acids.

B. General Procedure for pegylatingIgG and other Proteins with Alkyne-$(PEG)_n$-NHS

The degree of Alkyne-PEG-NHS ester incorporation can vary depending on the parameters of the pegylation reaction, including protein concentration, Alkyne-$(PEG)_n$-NHS 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 2 mg of IgG in 1mL of Buffer A: PBS (for example, 0.1M sodium phosphate 0.15M NaCl, pH 7.2).
2. Immediately before use, dissolve 1 mg of the Alkyne-$(PEG)_n$-NHS ester in 75 µL of DMF or DMSO. Add 25 µL of the Alkyne-$(PEG)_n$-NHS solution to the IgG solution.
3. Incubate the reaction on ice for two hours or at room temperature for 30 minutes.
4. Remove unreacted Alkyne-$(PEG)_n$-NHS by dialysis or gel filtration.
5. Store the alkyne-pegylated protein at the same conditions as specified for the unpegylated protein until ready for use.

For Click Chemistry 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).