Application Instructions for Y-PEG-NHS-40K for Amine PEGylation

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Introduction

The process of PEGylation, the covalent conjugation of a PEG derivative onto molecules, improves the water solubility and biocompatibility of those molecules, a beneficial feature for protein drug development [1]. The sterically bulky structure of JenKem Technology's proprietary Y-shaped branched PEG derivatives, consisting of two linear methoxy PEG chains attached to a central active core, may help to reduce the number of attachment sites to a protein molecule. As a PEG NHS ester, Y-shaped PEG NHS ester is readily dissolved in aqueous buffers and enables simple and efficient modification of proteins and other biological agents that contain lysines.

Compared to linear PEG NHS esters, under typical PEGylation conditions, the Y-shaped PEG NHS esters have increased selectivity towards more sterically available amines, due to their bulky structure [2-6]. Protein PEGylation with Y-shaped PEG NHS esters can be accomplished in less than one hour under the suggested reaction conditions.

Materials

Y-PEG-NHS-40K (Y-shaped PEG NHS Ester, MW 40000, Aldrich Prod. No. JKA0001)
Phosphate buffer or other amine-free buffer of your choice at pH 7.0-7.5
Protein to be PEGylated
Dry water-miscible solvent (e.g., dry DMF or DMSO)

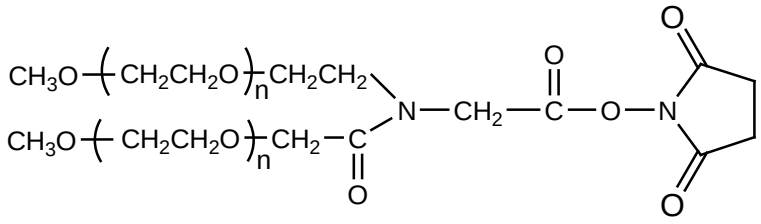

Figure 1. Chemical structure of Y-PEG-NHS-40K (Y-shape PEG NHS Ester, MW 40000, Aldrich Prod. No. JKA0001)

Method for Protein PEGylation with Y-PEG-NHS-40K

1. Sample Storage and Handling

All PEG derivatives should be stored under a dry inert gas (argon or nitrogen), in the dark, at or below -20°C.

In addition, PEG succinimidyl active esters, including Y-PEG-NHS-40K, will hydrolyze in water. It is essential to minimize exposure of these products to moisture and to keep them cold to reduce the degree of hydrolysis.

Before using a PEG derivative after storage, allow the bottle containing the reagent to warm to room temperature slowly. After using, the reagent container should be backfilled with a dry inert gas (argon or nitrogen) and store again at or below -20°C. For multiple uses, in order to avoid repeated cycling through multiple freeze-thaw cycles, it is a good practice to divide PEG derivatives into several small portions at the first use.

2. PEGylation Protocol using JenKem Technology's Y-NHS-40K

We describe here a general 5 step-protocol for initial studies of protein PEGylation using a Y-shape PEG NHS ester (Y-PEG-NHS-40K). As mentioned in the introduction, Y-PEG-NHS-40K reacts efficiently with primary amino groups on the side chain of lysines on protein at pH 7-7.5, forming amide bonds, along with releasing free NHS.

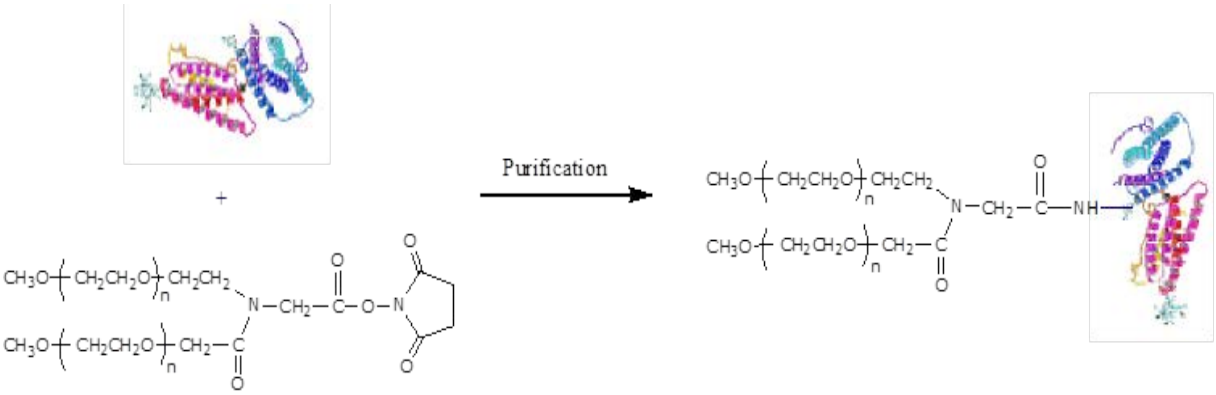

Figure 2. Schematic of protein PEGylation using a Y-PEG-NHS-40K

Step 1:
Take the bottle containing Y-PEG-NHS-40K from storage and fully equilibrate it to room temperature before use. Remember to seal under inert gas (argon or nitrogen), and keep at or below -20°C after use.

Step 2:
Calculate the amount (mole or mmole) of protein to be PEGylated and dissolve the protein in a phosphate buffer or other amine-free buffer of your choice at pH 7.0-7.5.

Proteins stored in Tris or other amine-containing buffers must be exchanged into a suitable buffer before use.

Step 3:

Depending on the nature of the protein and the degree of PEGylation desired, the amount of Y-PEG-NHS-40K to be used for each PEGylation process differs. As a starting point, consider using a 5- to 10- folds molar excess of Y-PEG-NHS for protein solutions concentration of not less than 2 mg/mL. When protein solution concentration is more dilute, a greater relative molar excess of Y-PEG-NHS-40K may be necessary for desired PEGylation results.

Calculate the amount of Y-PEG-NHS-40K to be used in PEGylation reaction, dissolve the Y-NHS-PEG-40K in dry water-miscible solvent (e.g., dry DMF or DMSO) and add slowly to the protein solution with a gentle swirl.

Step 4:
Incubate reaction mixture at 0-5ºC for about three hours, or at room temperature for about one hour.

Reaction time may vary with the nature of proteins. Longer reaction times may be used, however, monitor for protein degradation or microbial growth.

Step 5:
When PEGylation reaction is finished, the degree of PEGylation should be evaluated. Mono PEGylated protein can be purified from multiply PEGylated protein and non-PEGylated protein through chosen chromatographic methods.

References

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