Instructions for the use of endo-BCOT-(PEG)$_n$-PFP ester

Introduction

The BroadPharm **endo-BCOT-(PEG)$_n$-PFP ester** is a triple bond labeling reagent that reacts with primary and secondary amines. This cyclic triple bond reacts with the azide group simultaneously without needing of a metal catalyst; it is **non-metal catalyzed 'click' chemistry** functional group. The pentafluorophenyl (PFP) ester-activated triple bond PEG linker is less subject to hydrolysis than NHS esters,.endo-BCOT-(PEG)n-PFP ester must be first 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. Unlike many conjugation reagents' endo-BCOT and azide activated biomolecules have long-term stability. The endo-BCOT-azide conjugation reaction is a truly chemoselective and bioorthogonal ligation reaction that can be performed in a complex mixture of biological molecules in aqueous media without reacting with any of them.

Important Product Information

• Endo-BCOT-(PEG)$_n$-PFP ester is moisture-sensitive. Store the vial of reagent 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 reagent immediately before use. The PFP 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).

• After pegylation, the 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.

• General Procedure for pegylating IgG and other Proteins with endo-BCOT-(PEG)$_n$-PFP ester

The degree of endo-BCOT-(PEG)$_n$-PFP ester incorporation can vary depending on the parameters of the pegylation reaction, including protein concentration, endo-BCOT-(PEG)$_n$-PFP 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 1 mL of PBS (for example, 0.1M sodium phosphate 0.15M NaCl, pH 7.2).
2. Immediately before use, dissolve 1 mg of endo-BCOT-(PEG)$_n$-PFP ester in 75 µL of DMF or DMSO. Add 25 µL of the endo-BCOT-(PEG)$_n$-PFP solution to the IgG solution.
3. Incubate the reaction on ice for two hours or at room temperature for 30 minutes.

4. Remove unreacted reagent by dialysis or gel filtration.
5. Store the pegylated protein at the same conditions specified for unpegylated protein until ready for use.

• General Procedure for Chemoselective Ligation through Copper-free Click Chemistry

Our conjugation chemistry is based on the reaction of the endo-BCOT-$(PEG)_n$-PFP ester reagent with an azide linker to form a stable triazole. The 'click reaction' is very fast at room temperature, it doesn't require a cytotoxic Cu(I) catalyst and creates a stable triazole. This unique covalent bond is created when endo-BCOT, incorporated into one type of biomolecule reacts with an azide linker, incorporated into a second biomolecule.

This method requires a three-step reaction:

Step 1: Activation of Biomolecule #1 with endo-BCOT-$(PEG)_n$-PFP ester linker

Step 2: Activation of Biomolecule #2 with azide

Step 3: Mixing the two activated biomolecules to form a conjugate

Step 4: (optional): Removal of the excess of azide or endo-BCOT activated biomolecule with endo-BCOT or an azide scavenger

Product Features and Benefits:

• Stable: forms a triazole

• Biocompatabile:catalyst not required (e.g. Cu(I))

• Specificity: azide reacts only with endo-BCOT in the presence of $-NH_2$, -SH,-COOH and other protein functionalities

• The reactive moieties do not interact with functionalities on biomolecules

• The chemistry is all done in aqueous buffered media, yielding high efficiency conjugation

Important endo-BCOT Product Information:

• Avoid buffers that contain azides, which can react with the endo-BCOT.

• Reactions with endo-BCOT and azides are more efficient at high concentrations and temperatures (i.e., 4- 37°C).

• Typical reaction times are less than 2 hours; however, incubating for longer can improve efficiency.

General Protocol:

Copper-free Click Reaction

1. Prepare the azide□containing protein in reaction buffer.

2. Add endo-BCOT□bio molecule to azide□containing sample.

Recommendation: Add 1 mol equivalent of limiting reagent to 1.5-2 mol equivalents of the highest abundance reagent.

3. Incubate the reaction at room temperature for 2-6 hours or at 4 ºC for 6-12 hours.

4. The reaction is now ready for purification by size exclusion chromatography if desired.