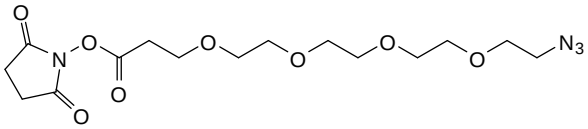
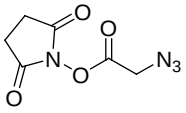
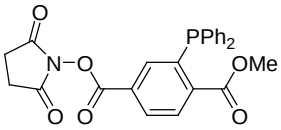
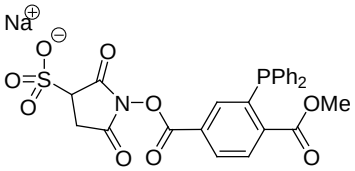
INSTRUCTIONS

NHS-Azide and NHS-Phosphine Reagents

26130 88900 88902 88906

2199.4

Number	Description	
26130	**NHS-PEG$_4$-Azide**, 100mg Molecular Weight: 388.37	
88902	**NHS-Azide**, 10mg Molecular Weight: 198.14	
88900	**NHS-Phosphine**, 10mg Molecular Weight: 461.40	
88906	**Sulfo-NHS-Phosphine**, 10mg Molecular Weight: 563.45	

Storage: Upon receipt store at -20°C protected from light and moisture. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific™ NHS-Azide and NHS-Phosphine Reagents are amine-reactive compounds for derivatizing primary amines of proteins or amine-coated polymer surfaces. Once a protein or surface is azide- or phosphine-labeled, the two components are mixed for effective and stable conjugation. Phosphine groups react with azides via a Staudinger reaction to produce an aza-ylide intermediate that is trapped to form a stable, covalent amide bond (Figure 1).[1] Because phosphines and azides are absent from biological systems, there is minimal background labeling of macromolecules found in cells or lysates.[2]

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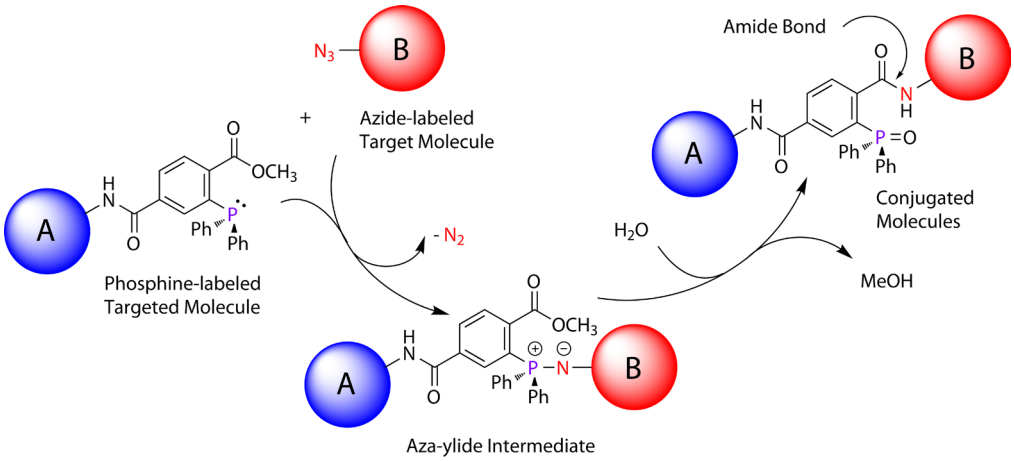

Figure 1. Staudinger ligation reaction scheme (azide-phosphine conjugation). Azide and phosphine reagent pairs enable specific crosslinking and labeling of biomolecules to facilitate cellular pathway analysis. When combined, phosphine-activated compounds conjugate with high specificity to azide-tagged molecules, resulting in stable covalent attachment of "A" and "B" molecules.

Important Product Information

- NHS esters are moisture-sensitive. To avoid moisture condensation onto the product, equilibrate vial to room temperature before opening. The NHS-ester moiety readily hydrolyzes and becomes non-reactive; therefore, prepare stock solutions immediately before use.
- Hydrolysis of the NHS ester is a competing reaction. Conjugation with primary amines of proteins/peptides (i.e., acylation) is favored at near neutral pH (6-9) and with concentrated protein solutions. For conjugation, use non-amine-containing buffers at pH 7-9 such as PBS (20mM sodium phosphate, 150mM sodium chloride, pH 7.4; Product No. 28372); 20mM HEPES; 100mM carbonate/biocarbonate; or 50mM borate buffer. Do not use buffers that contain primary amines, (e.g., Tris, glycine), which compete with acylation.
- If possible, avoid reducing agents in reaction buffers, which can interfere with azide stability.
- Reactions with phosphines and azides are more efficient at high concentrations and temperatures (i.e., 23-37°C). Typical reaction times are less than 4 hours; however, incubating for longer can improve efficiency.
- NHS-PEG_4-Azide contains a polyethylene glycol (PEG) spacer arm. The hydrophilic PEG spacer arm confers greater solubility to the rest of the molecule and the labeled compound. However, NHS-PEG_4-Azide does not easily dissolve directly in water or aqueous buffer. Dissolve the reagent in a dry water-miscible organic solvent such as DMSO or DMF for storage as a concentrated stock (see Procedure).
- Dissolve NHS-Azide in a dry water-miscible organic solvent such as DMSO or DMF before diluting in final reaction buffer. NHS-Azide is soluble in aqueous buffers up to 5mM.
- Dissolve NHS-Phosphine and Sulfo-NHS-Phosphine in a dry water-miscible organic solvent such as DMSO or DMF before diluting in final reaction buffer. NHS-Phosphine is not soluble in aqueous buffers. Sulfo-NHS-Phosphine is soluble in aqueous buffers up to 10mM.

Procedure for Protein Conjugation

The following protocol is an example application for this product. Specific applications will require optimization.

A. Additional Materials Required

- Dry dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF)
- Phosphate-buffered saline (PBS; 20mM sodium phosphate, 0.15M sodium chloride; pH 7.2, Product No. 28372)
- Quenching Buffer: 1M Tris•HCl, pH 8.0
- Thermo Scientific™ Zeba™ Spin Desalting Columns or Thermo Scientific™ Slide-A-Lyzer™ Dialysis Cassettes

B. Protein Derivatization

1. Prepare proteins in PBS, with a protein concentration ≥ 5mg/mL.
2. Immediately before use, prepare 10mM of the NHS reagent:
   - For NHS-Phosphine (Product No. 88900), dissolve 1mg reagent in 217µL of DMSO or DMF.
     For Sulfo-NHS-Phosphine (Product No. 88906), dissolve 1mg reagent in 177µL of DMSO or DMF.
   - For NHS-Azide (Product No. 88902), dissolve 1mg reagent in 505µL of DMSO or DMF.
   - For NHS-PEG_4-Azide (Product No. 26130), whose consistency is not amenable to weighing small quantities, prepare a 10X (100mM) stock solution by dissolving the entire contents (100mg) of reagent in 2.57mL of dry (moisture-free) DMSO or DMF for storage in aliquots at -20°C. Equilibrate aliquot to room temperature before opening to avoid condensation, then use it promptly. Stock solutions that absorb moisture will hydrolyze.
3. Add the NHS reagent to the protein sample at a final concentration of 0.5-2mM. If the protein concentration is ≥ 5mg/mL, use a 10-fold molar excess of the reagent. For samples < 5mg/mL, use a 20- to 50-fold molar excess.
4. Incubate the reaction at room temperature for 30 minutes or on ice for 2 hours.
5. Stop the reaction by adding Quenching Buffer to a final concentration of 50-100mM Tris.
6. Incubate the reaction at room temperature for 5 minutes or on ice for 15 minutes.
7. Remove excess non-reacted NHS-ester using a Zeba Spin Desalting Column or Slide-A-Lyzer Dialysis Cassette.

C. Protein Conjugation

1. Mix equal molar amounts of the azido-derivated and phosphine-derivated proteins and incubate at 37°C for 2-4 hours. Room temperature or 4°C incubation requires 16-24 hours. The final concentration of each protein should be ≥ 2mg/mL.
2. To isolate conjugate from the two unconjugated proteins, use an appropriate size exclusion chromatography method.

Procedure for Protein Azide Biotinylation

The following protocol is an example application of protein labeling using Phosphine-PEG_3-Biotin (Product No. 88901).

Additional Materials Required

- Same materials as in Procedure A

Azide Biotinylation

1. Prepare the azide-containing protein sample in reaction buffer following Procedure B.
2. Dissolve 1mg of Phosphine-PEG_3-Biotin with 126µL of DMSO or DMF to make 10mM solution. Store DMSO stock solutions at -20°C for up to 6 months.
3. Add Phosphine-PEG_3-Biotin to a final concentration of 50-200µM to the protein sample. If the protein concentration is ≥ 5mg/mL, use a 10-fold molar excess of the reagent. For samples < 5mg/mL, use a 20-fold molar excess.
4. Incubate the reaction at 37°C for 2-4 hours. Room temperature incubation requires 16-24 hours.
5. Remove excess non-reacted Phosphine-PEG_3-Biotin using a desalting column or dialysis cassette.

Troubleshooting

Problem	Possible Cause	Solution
No conjugation of phosphine with azide	One or more proteins not labeled	Confirm molecules were labeled or repeat activation process
	NHS-ester hydrolyzed	Allow product to equilibrate to room temperature before opening
		Prepare new solutions in the indicated dry solvents
		Avoid buffers that contain primary amines such as Tris and glycine
	Excess reagent not quenched or removed	Remove non-reacted reagent by dialysis or desalting
Low conjugation of phosphine and azide	Suboptimal reaction conditions	Optimize conjugation conditions by altering molar excess
		Perform conjugation reactions at 37°C
		Increase incubation time

Related Thermo Scientific Products

88901 **Phosphine-PEG$_3$-Biotin**, 10mg

88903 **GlcNAz** (*N*-azidoacetylglucosamine, tetraacylated), 5mg

88904 **ManNAz** (*N*-azidoacetylmannosamine, tetraacylated), 5mg

88905 **GalNAz** (*N*-azidoacetylgalactosamine, tetraaceylated), 5mg

88907 **DyLight™ 488-Phosphine**, 1mg

88910 **DyLight 550-Phosphine**, 1mg

88911 **DyLight 650-Phosphine**, 1mg

28372 **BupH™ Phosphate Buffered Saline Pack,** 40 packs

66382, 66807 **Slide-A-Lyzer Dialysis Cassette Kits**, for 0.5-3mL and 3-12mL sample volumes, respectively

89889, 89891 **Zeba Spin Desalting Columns**, for 200-700µL samples and 600-2000µL sample volumes, respectively

Cited Reference

1. Saxon, E. and Bertozzi, C. (2000). Cell surface engineering by a modified Staudinger reaction. *Science* **287:**2007-10.
2. Agard, N., *et al.* (2006). A comparative study of bioorthogonal reactions with azides. *ACS Chemical Biology* **1(10):**644-8.