

EZ-Link[®] Phosphine-PEG₃-Biotin

88901

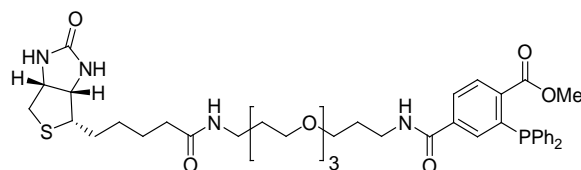
2200.1

Number 88901

Description EZ-Link Phosphine-PEG₃-Biotin, 10mg

Molecular Weight: 792.92

Spacer Arm: 33Å



Storage: Upon receipt store at -20°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific EZ-Link Phosphine-PEG₃-Biotin is a versatile biotinylation reagent for labeling azide-containing molecules. The phosphine group reacts with an azide to produce an aza-ylide intermediate that is trapped to form a stable, covalent amide bond (Figure 1), which is also referred to as the Staudinger reaction.¹ Because phosphines and azides are absent from biological systems, there is minimal background labeling of cells or lysates.² Labeled proteins can be purified using immobilized streptavidin, avidin or Thermo Scientific NeutrAvidin Protein affinity resins and detected in ELISA, dot blot or Western blot applications.

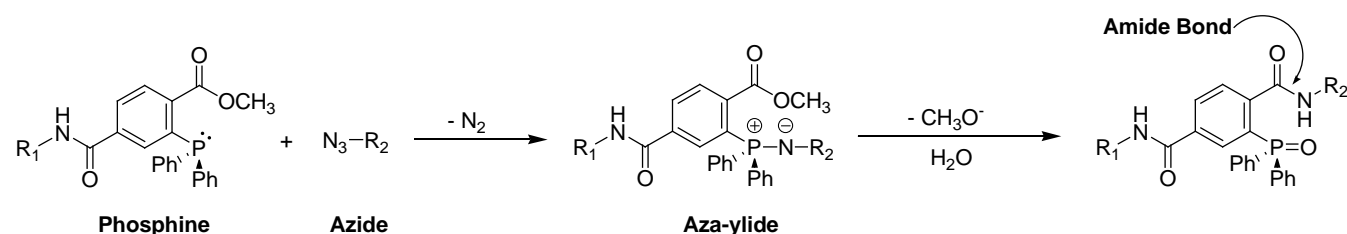


Figure 1. Reaction scheme of azide-containing molecules, which is also referred to as the Staudinger reaction.

Important Product Information

- 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.
- Dissolve EZ-Link Phosphine-PEG₃-Biotin in a dry water-miscible organic solvent such as DMSO or DMF before diluting in final reaction buffer. EZ-Link Phosphine-PEG₃-Biotin is soluble in aqueous buffers up to 0.5mM.

Procedure for Azide Biotinylation

A. Additional Materials Required

- Water-miscible organic solvent such as dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF)
- Reaction buffer: Phosphate-buffered saline (PBS) or other buffer at pH 6-8
- Thermo Scientific Zeba Spin Desalting Columns or Slide-A-Lyzer Dialysis Cassettes

A. Azide Biotinylation

1. Prepare the azide-containing protein sample in reaction buffer.
2. Dissolve 1mg of EZ-Link Phosphine-PEG₃-Biotin with 126μL of DMSO or DMF to make 10mM. Store DMSO stock solutions at -20°C for up to 6 months.
3. Add EZ-Link Phosphine-PEG₃-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 EZ-Link Phosphine-PEG₃-Biotin using a desalting column or dialysis cassette.

Troubleshooting

Problem	Possible Cause	Solution
Low biotinylation efficiency	Suboptimal reaction conditions	Optimize conjugation conditions by altering molar excess of phosphine to azide
		Perform conjugation reactions at 37°C
		Increase incubation time

Related Thermo Scientific Products

88902	NHS-Azide, 10mg
88900	NHS-Phosphine, 10mg
88906	Sulfo-NHS-Phosphine, 10mg
88903	GlcNAz (<i>N</i> -azidoacetylglucosamine, tetraacylated), 5mg
88904	ManNAz (<i>N</i> -azidoacetylmannosamine, tetraacylated), 5mg
88905	GalNAz (<i>N</i> -azidoacetylgalactosamine, tetraacylated), 5mg
88907	DyLight® 488-Phosphine, 1mg
88910	DyLight 550-Phosphine, 1mg
88911	DyLight 650-Phosphine, 1mg
28372	BupH™ Phosphate Buffered Saline Pack, 40 packs
21126	Streptavidin, Horseradish Peroxidase Conjugated, 1mg
20357	High Capacity Streptavidin Agarose Resin, 2mL
89889	Zeba Spin Desalting Columns, 7K MWCO, 2mL

Cited References

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.

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Current product instructions are available at www.thermoscientific.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

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