

Biotinylate carboxyl groups with EDC and Biotin Hydrazide

TR0015.2

Introduction

This Tech Tip describes a procedure for biotinylating a molecule through carboxylic acid groups using selected Thermo Scientific Pierce Protein Research Products. EZ-Link[®] Biotin Hydrazide compounds are typically used for labeling oxidized carbohydrate moieties, but their terminal amino groups can be used effectively with the water-soluble carbodiimide EDC to label carboxylates. EDC interacts with carboxyl groups, making them reactive to the hydrazide amino groups. EDC reactions are usually performed in acidic buffer (pH 4.7-5.5), but efficient coupling can be accomplished in amine- and carboxylate-free buffers at pH values up to pH 7.4.

When labeling proteins with biotin, researchers most commonly choose reagents that target primary amino groups, which are available in the side chain of lysine residues and at the N-terminus of each polypeptide. *N*-hydroxysuccinimidyl esters (NHS esters) of biotin are the reagents of choice for amine-targeted labeling (e.g., Sulfo-NHS-LC-Biotin, Product No. 21335). However, amines are not the only available target for labeling on proteins, and situations exist in which labeling through amines adversely affects protein function. In such cases, labeling through carboxyl groups may result in a more successfully (i.e., active) labeled molecule. Alternatively, amino groups may not be available on a molecule, either because they are not present or because they have been targeted in a previous labeling or crosslinking experiment.

Be aware that the EDC/hydrazide reaction involves conjugation between carboxylates and amines, both of which are present in most proteins; consequently, protein polymerization may occur unless a large molar excess of biotin hydrazide (over other amines) is included in the reaction. Some optimization will be necessary. Alternatively, amines on the protein may be blocked irreversibly using Sulfo-NHS-Acetate (Product No. 26777) before performing the EDC/hydrazide reaction.

Materials Required

- Protein (molecule to be labeled): This protocol assumes use of a moderately-sized protein (30-100 kDa) containing several amino and carboxyl groups.
- Reaction Buffer: 0.1 M MES (*N*-morpholinoethane sulfonic acid) buffer, pH 4.7-5.5 (Product No. 28390); alternatively, phosphate or other buffer (pH 4.5-7.4) that is free of amines and carboxylates may be used.
- Biotin Hydrazide: Choose either Biotin Hydrazide (Product No. 21339), Biotin LC-Hydrazide (Product No. 21340) or Biotin-PEG₄-Hydrazide (Product No. 21360)
- EDC (Product No. 22980)
- Zeba[™] Desalt Spin Column (e.g., Product No. 89891) or Slide-A-Lyzer[®] Dialysis Cassette (e.g., Product No. 66382)

Biotinylation Protocol

1. Dissolve 5-10 mg of Protein in 1 ml Reaction Buffer.
2. Prepare a solution of 50 mM Biotin Hydrazide in dry DMSO (e.g., 18.5 mg Biotin LC-Hydrazide in 1 ml DMSO).
3. Add 25 μ l of the Biotin Hydrazide solution to the Protein solution and mix. (Results in 1.25 mM Biotin Reagent.)
4. Immediately before use, prepare 500 mM EDC in Reaction Buffer. (Dissolve 10 mg EDC in 0.1 ml Reaction Buffer.)
5. Add 12.5 μ l of the EDC solution to the Protein/Biotin Hydrazide solution from Step 3. (Results in ~5 mM EDC.)
6. Incubate reaction for 2 hours to overnight at room temperature.
7. Remove any precipitate (polymers of the protein) that formed during the reaction by centrifugation.
8. Remove non-reacted Biotin Hydrazide and EDC byproducts from the labeled protein by desalting or dialysis.

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

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