

# Preferentially biotinylate N-terminal $\alpha$ -amino groups in peptides

TR0046.1

## Introduction

Biotinylation reagents containing *N*-hydroxysuccinimide (NHS) esters are widely used to label proteins at primary amino groups (-NH<sub>2</sub>), which exist in the side chain of lysine residues and at the N-terminus of each polypeptide. With large proteins, labeling of several lysine residues and the N-terminus does not usually harm protein function or binding properties. With short peptides, however, the random biotinylation of the  $\epsilon$ -amino groups of lysine residues is much more likely to block binding sites whose function is necessary for downstream applications of the biotinylated peptide. Labeling of lysine residues in peptides, especially short ones, may alter their functional properties and/or immunoreactivity.

The protocol described below allows for the selective biotin-labeling of the N-terminal  $\alpha$ -amino group of short peptides and is based on the method described by S elo, *et al.*<sup>1</sup> Such preferential labeling is achieved by using a reaction pH that is lower than the typical range used for reaction by NHS-ester reagents. Because the p*K*<sub>a</sub> of the  $\alpha$ -amino group (p*K*<sub>a</sub> = 8.9) is considerably lower than that of the  $\epsilon$ -amino group of lysine (p*K*<sub>a</sub> = 10.5), maintaining a lower-than-normal reaction pH ensures that the lysine amines are very rarely in the unprotonated state that allows them to react. Optimal pH, reagent concentration, and reaction time may have to be determined experimentally for each particular peptide.

## Materials Required

- Reaction Buffer: 50 mM phosphate (pH 6.5) or other non-amine containing buffer at pH 6.5.
- NHS-Biotin Reagent: NHS-LC-Biotin (Product No. 21336). Other NHS or Sulfo-NHS Biotin Reagents will likely work just as well in this application. See our web site for a complete and current listing.
- Size-exclusion separation method for removal and separation of non-reacted biotin and byproducts from the labeled peptide. Desalting columns and dialysis units can be used if they have resins or membranes with molecular weight cut-off (MWCO) values less than the peptide MW. Otherwise, HPLC is necessary to efficiently separate small peptides from non-reacted biotin reagent and byproducts.

## Example Protocol

1. Dissolve 1-10 mg of peptide in 1 ml Reaction Buffer. DMF or DMSO may be used to dissolve the peptide initially; however, keep the final concentration of the organic solvent at <20% in the Reaction Buffer.
2. Immediately before use, prepare a 10 mM stock solution of NHS-LC-Biotin in DMF or DMSO (4.5 mg NHS-LC-Biotin in 1 ml of solvent yields 10 mM).
3. Add sufficient volume of 10 mM NHS-LC-Biotin to achieve a 5-fold molar excess of biotin over the peptide in solution.
4. Incubate the reaction mixture at 4 °C for 24 hours.
5. Use dialysis, gel filtration or HPLC to remove non-reacted NHS-LC-Biotin and reaction byproducts (see instructions provided with preferred buffer exchange product).
6. Store the biotinylated peptide in the same manner as was appropriate for the non-biotinylated peptide.

## Reference

1. S elo, I., *et al.* (1996). Preferential labeling of  $\alpha$ -amino N-terminal groups in peptides by biotin: application to the detection of specific anti-peptide antibodies by enzyme immunoassays. *J. Immunol. Methods* **199**:127-38.

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