

NHS-Biotin Protocol and Product Information Sheet

Product Category: Biotinylation Reagents

Catalog Number(s): <u>b2101-100mg</u>, <u>b2101-1gm</u>, b2101-custom

Product Name: NHS-Biotin

Alternative Name(s): Succinimidyl biotin; Succinimidobiotin

CAS Number: 35013-72-0 Chemical Formula: $C_{13}H_{19}N_3O_4S$ Molecular Weight: 341.38 Spacer Length: 13.5 Å

Storage: Upon receipt store at 4°C (shipped at ambient temperature).

General NHS-Biotin Protein Biotinylation Protocol

- 1. Allow vial of NHS-Biotin to equilibrate to ambient temperature before opening.
- 2. Dissolve protein at a concentration of 1-10 mg/mL in 100 mM sodium phosphate, 150 mM NaCl, pH 7.2-7.5 or other suitable amine-free buffer.
- Immediately before use, create a 20 mg/mL NHS-Biotin stock solution in anhydrous DMF (<u>cr8106-25ml</u>) or DMSO (<u>cr8105-25ml</u>).
- 4. Add sufficient NHS-Biotin stock solution to the protein solution to obtain 10-20 fold molar excess of biotinylation reagent over protein.

Note: Dilute protein solutions (i.e. 1-2 mg/mL) may require increased molar excess of NHS-Biotin (i.e. \geq 20 fold) to yield similar biotinylation of a more concentrated protein solution.

- 5. Allow biotinylation reaction to proceed for 30-60 minutes at room temperature or \geq 2 hours at 4°C.
- 6. Desalt biotinylated protein through dialysis or gel filtration with a resin, such as Sephadex® G-25 (g4109) or equivalent.

References:

Hermanson, G.T. 1996. Bioconjugate Techniques. Academic Press, San Diego, CA, USA.