

NHS-LC-Biotin Protocol and Product Information Sheet

Product Category: Biotinylation Reagents

Catalog Number(s): <u>b2102-100mg</u>, <u>b2102-1gm</u>, b2102-custom

Product Name: NHS-LC-Biotin

Alternative Name(s): Succinimidyl 6-(biotinamido)hexanoate; (+)-Biotinamidocaproate N-

hydroxysuccinimidyl ester

CAS Number: 72040-63-2 Chemical Formula: $C_{20}H_{30}N_4O_6S$ Molecular Weight: 454.54 Spacer Length: 22.4 Å

Storage: Upon receipt store at -20°C (shipped at ambient temperature). Protect

from moisture (i.e. humidity); blanket under desiccated inert gas.

General NHS-LC-Biotin Protein Biotinylation Protocol

- 1. Allow vial of NHS-LC-Biotin to fully equilibrate to ambient temperature before opening to prevent condensation inside the vial (NHS-LC-Biotin is moisture-sensitive).
- 2. Allow vial of NHS-LC-Biotin to equilibrate to ambient temperature before opening.
- 3. Dissolve protein at a concentration of 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 40 mg/mL NHS-LC-Biotin stock solution in anhydrous DMF (<u>cr8106-25ml</u>) or DMSO (<u>cr8105-25ml</u>).
- 5. Add sufficient NHS-LC-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-LC-Biotin (i.e. > 20 fold) to yield similar biotinylation of a more concentrated protein solution.

- 6. Allow biotinylation reaction to proceed for 30-60 minutes at room temperature or > 2 hours at 4°C.
- 7. 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.