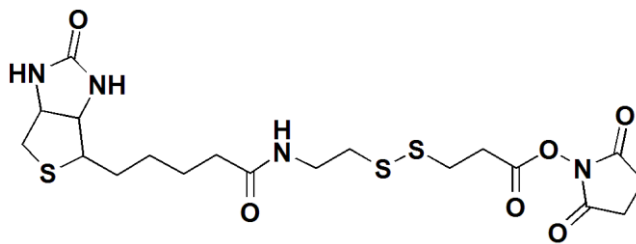


NHS-SS-Biotin Protocol and Product Information Sheet

Product Category:	Biotinylation Reagents
Catalog Number(s):	b2105-100mg , b2105-1gm , b2105-custom
Product Name:	NHS-SS-Biotin
Alternative Name(s):	Succinimidyl-2-(biotinamido)-ethyl-1,3'-dithiopropionate; Biotin disulfide N-hydroxysuccinimide ester
CAS Number:	142439-92-7
Chemical Formula:	C ₁₉ H ₂₈ N ₄ O ₆ S ₃
Molecular Weight:	504.65
Spacer Length:	24.3 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-SS-Biotin Protein Biotinylation Protocol

1. Allow vial of NHS-SS-Biotin to fully equilibrate to ambient temperature before opening to prevent condensation inside the vial (NHS-SS-Biotin is moisture-sensitive).
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.
3. Create a 5 mg/mL NHS-SS-Biotin stock solution in anhydrous DMF ([cr8106-25ml](#)) or DMSO ([cr8105-25ml](#)). This solution is stable at room temperature for 1-2 days.
4. Add sufficient NHS-SS-Biotin stock solution to the protein solution to obtain 10-20 fold molar excess of biotinylation reagent over protein (do not exceed 10% organic solvent volume in the final reaction).

Note: Dilute protein solutions (i.e. 1-2 mg/mL) may require increased molar excess of NHS-SS-Biotin (i.e. ≥ 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 ≥ 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.
7. To reverse the biotin tag, through reduction of the disulfide linkage, incubate biotinylated sample with 50 mM DTT ([cr8101-5x10mg](#)) or TCEP-HCl ([cr8104-1gm](#)) for 90-120 minutes at room temperature or 1 hour at 45°C.

References:

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