

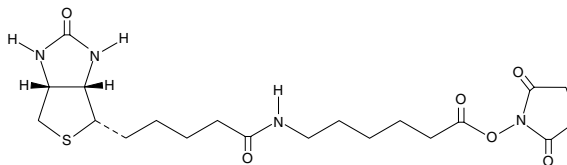
# Product Information



## Biotin-X-NHS

Item No. 13316

**CAS Registry No.:** 72040-63-2  
**Formal Name:** 6-[[5-[(3aS,4S,6aR)-hexahydro-2-oxo-1H-thieno[3,4-d]imidazol-4-yl]-1-oxopentyl]amino]-2,5-dioxo-1-pyrrolidinyl ester-hexanoic acid  
**MF:** C<sub>20</sub>H<sub>30</sub>N<sub>4</sub>O<sub>6</sub>S  
**FW:** 454.4  
**Purity:** ≥95%  
**Stability:** ≥2 years at -20°C  
**Supplied as:** A crystalline solid



For long term storage, we suggest that biotin-X-NHS be stored as supplied at -20°C. It should be stable for at least two years.

Biotin-X-NHS is supplied as a crystalline solid. A stock solution may be made by dissolving the biotin-X-NHS in the solvent of choice. Biotin-X-NHS is soluble in organic solvents such as DMSO and dimethyl formamide, which should be purged with an inert gas. The solubility of biotin-X-NHS in these solvents is approximately 20 mg/ml.

Biotin-X-NHS is sparingly soluble in aqueous buffers. For maximum solubility in aqueous buffers, biotin-X-NHS should first be dissolved in DMSO and then diluted with the aqueous buffer of choice. Biotin-X-NHS has a solubility of approximately 0.5 mg/ml in a 1:1 solution of DMSO:PBS (pH 7.2) using this method. We do not recommend storing the aqueous solution for more than one day.

N-Hydroxysuccinimide (NHS) activates carboxylic acid groups (on biotin) to facilitate coupling reactions. Biotin-X-NHS is a compound used to attach biotin to primary amines under alkaline conditions (pH-8-9). For example, lysines on the surface of proteins, including antibodies, are ideal targets for biotinylation with this compound. Biotin-X-NHS is like Biotin-NHS (Cayman No. 13315), but contains a six-atom spacer arm to minimize steric effects.

### Laboratory Procedures

Avoid performing biotinylation reactions in buffers containing primary amines (such as Tris). The primary amines in the buffers will compete with the protein for biotinylation. Prior to biotinylation, exchange the buffer to one which does not contain primary amines (such as potassium phosphate). This can be accomplished by dialysis or by running the protein solution over a desalting column. The optimal level of biotinylation will be protein dependent. The procedure below uses a twenty-fold molar excess of NHS-biotin relative to protein, which is appropriate for many proteins. If you are unsure if this level of biotinylation is appropriate for your protein, it may be advisable to set up a number of small biotinylation reactions, increasing and decreasing the ratio of biotinylation reagent to protein by four-fold (i.e., using molar excesses of NHS-biotin to protein of five-fold, twenty-fold and eighty-fold).

1. Dilute protein in 100 mM potassium phosphate, pH 7.4 to a final concentration of 0.5-10 mg/ml.
2. Just prior to use, prepare a 20 mM stock solution of NHS-biotin in dimethyl formamide or DMSO. This solution may be stored at -20°C for up to two weeks; long term storage is not recommended.
3. Add a twenty-fold molar excess of NHS-biotin to the protein.
4. Incubate one hour at room temperature, or overnight at 4°C.
5. Unreacted NHS-biotin may be quenched by the addition of an excess of 1 M Tris, pH 8.0. Alternatively, unreacted NHS-biotin may be removed by dialysis, or purifying with a desalting column.
6. The biotinylated protein is now ready for use.

### Related Products

Biotin-XX hydrazide - Cat. No. 13304 • Biotin-NHS - Cat. No. 13315

**WARNING: THIS PRODUCT IS FOR LABORATORY RESEARCH ONLY: NOT FOR ADMINISTRATION TO HUMANS. NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.**

#### MATERIAL SAFETY DATA

This material should be considered hazardous until information to the contrary becomes available. Do not ingest, swallow, or inhale. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. This information contains some, but not all, of the information required for the safe and proper use of this material. Before use, the user must review the complete Material Safety Data Sheet, which has been sent *via* email to your institution.

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