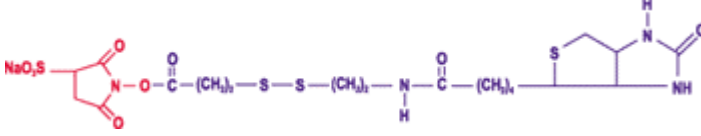


sNHS-SS-Biotin

Product Description

Catalog number:	UP53031A	100mg	UP53031B	50mg
Name :	Sulfosuccinimidyl-6-(biotinamido)ethyl-1,3-dithiopropionate (sNHS-SS-Biotin)			
Formula :				M.W.=606.7
State / Pack. :	Powder under nitrogen			
Storage :	-20°C			

General Considerations

The biotin is a vitamin widely used in biotechnology for its propriety to bind with extremely high affinity to avidin ($K_a=10^{-15} \text{ M}^{-1}$) and streptavidin ($K_a=10^{-14} \text{ M}^{-1}$). This interaction hapten-protein resists effectively to drastic physico-chemical conditions, allowing various immuno-technologies. The biotin can be conjugated through several chemical reactions to molecules of interest, notably proteins, without modifying the biological activity of the molecule, thanks to its low MW / volume and hydrophyllicity.

Interchim offers high quality biotins activated by the succinimidyl ester, easy to use in labs, rendering these 'NHS-biotins' very usefull and versatile biotinylation agents of proteins (antibodies). This technical sheet covers 'cleavable NHS-biotin', reversible sensitive labelling

After a biomolecule has been biotinylated, it is easily detected thanks to labelled (strept)avidins, allowing very **sensitive detections**. Then, it is possible to dissociate the biotin label, for subsequent other detections. It also has been used to biotinylate surfaces ([Fritzsche 1998](#)). An other main application is the capture of biomolecules from complex mixtures by avidin supports (UP39090) and further elution for **purification purposes** with protein/ligand complexes (protein/hormones, protein/DNA).

Scientific and Technical Information

- The chemical group N-hydroxysuccinimydyl (NHS) reacts in aqueous phase on primary ($-\text{NH}_2$) and secondary amines ($=\text{NH}$) (in fact on its deprotonated form), optimally at neutral pH or higher : amines present in proteins (Lys aminoacid) and in a lower proportion on NH_2 located in terminal peptidic chains. The reaction accomadates +37 to +4°C temperatures, and undergo in minutes to several hours. It competes with hydrolysis, that increases with pH, and with the high dilutions of the molecule that should be biotinylated. The concentration of incubation depends on the protein concentration, and desired biotinylation level; usual ratios are 2 to 20 NHSbiotin per amine (peptides) or 30-300 μmol per mg of protein.
- The sulfonyl moiety (NaSO_3) introduces a hydrophilic group that allows the product not to cross biological membranes. This is particularly useful to label, in situ on cells, proteins presented outside membranes, and if one wants to avoid the biotinylation of intracellular proteins that may affect further analysis, or may affect the cell metabolism. An other interest of the sulfonyl group is to permit the solubilisation of the product directly in aquous buffers, up to 10mM, avoiding the use of organic solvents like DMSO or DMF, that are possibly nocive to cells or applications.

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- The link between biotin and NHS is 24.3Angstroms. This spacer arm favorize the availability of Biotin for (strept)avidin, hence finally increases th sensitivity of detection. It contains a dissulfide bridge (S-S) that is cleavable by reducing agents in mild conditions. Usual agents are β -mercaptoethanol, DTT (#UP284250), and TCEP (#UP242214). Cleavable conditions are usually achieved with 10 μ M of reducing agent at room temperature. Glutathion might be helpful to reduce while maintaining physiological conditions, as it do not penetrate in cells (p), as well as TCEP a membrane impermeant potent and odorless reducer.

Directions for Use

Main directions for use and scientific information are similar to classic NHS biotins (see FT-UP52117).

Modifications of following protocoles may be needed to optimize the biotinylation level for each protein, for each cell type, or in any other application.

- **SulfoNHS-Biotin** can be dissolved in distilled water or added directly to the proteic solution (buffer of biotinylation) up 10mM. Uptima recommends not to store the stock solution, because the product is readily subject to hydrolysis. A limited storage may be obtained when using high quality anhydrous DMSO under argon or nitrogen gas at -20°C.
- The possible conditions of the esterification reaction are various. The biotinylation is usually realised in a neutral buffer, like PBS (NaCl 150mM, phosphate 20mM, pH7.4), or carbonate. The buffer should be free of amines (no Tris) and free of reducing agents (as DTT).
- It is usually necessary to remove by-products after labelling (excess of sNHS-SS-biotin, free biotin and NHS) by dialysis (CelluSep), gelfiltration columns (UP84874), or other suitable mean.

Protocol 1 : biotinylation of an antibody

This simple and quick standard protocole biotinylates polyclonal and monoclonal purified antibodies for immunodetection applications. It suits also most proteins and peptides if a similar concentration weight of NHS-Biotin / weight of protein or peptide is observed.

- 1- Prepare the antibody at 5mg/ml in PBS (NaCl 150mM, Phosphate 20mM, pH7.5). This can be done by dissolving the lyophilized antibody, or by dilution. Check if it contains no other proteins or Tris or other interfering agents. If not, purify, dialyse, or gelfiltrate in the right buffer. Other concentrations can be realised, but the coupling ratio should be slightly increased if the antibody is more diluted.
- 2- Prepare a sNHS-SS-biotin solution at 20mM in distilled water or in anhydrous DMSO.
- 3- Add 15 μ L of the solution of sNHS-SS-biotin to the antibody (1ml).
Incubate 1H at room temperature.
- 4- Dialyses the antibody against PBS+NaN3 0.01% (Use CelluSep membranes). The biotinylated antibody can be diluted to 1mg/ml with 0.1% NaN3 and 20% of glycerol for storage at -20°C or +4°C.

The level of biotinylation can be estimated by quantification of biotins (or for high biotinylation rates, by a differential quantitation of amines).

The conditions of biotinylation, ratio of NHS-biotin / molecule, temperature, duration of incubation, procedure of purification, can be adapted depending on quantity, volume or concentration of antibody, desired biotinylation degree, or on the susceptibility of the antibody (notably monoclonals can be partially inactivated). The optimization of the biotinylation level is classically determined by incubating the antibody with ratios of NHS-biotin / protein below and above the standard ratio, then by testing the biotinylated antibodies directly in the application to select the best result.

Protocole 2 : biotinylation of a peptide

This simple and rapid standard protocol, biotinylates peptides preferentially on Lys residues, but a biotinylation of the terminal NH2 is possible with higher ratios of sNHS-biotin / peptide at lower pH:

- 1- Prepare the peptide at 5mM in PBS (NaCl 150mM, Phosphate 20mM, pH7.5), usually by dissolving the lyophilised peptide. Do not use Tris based buffers.
- 2- Prepare a solution of sNHS-SS-biotin at 50mM in distilled water or in anhydrous DMSO.
- 3- Add 200-400 μ l of sNHS-SS-biotin in 1 ml of peptide solution at 5mM
Rem : add up to 4 other times 200 μ l at 15min intervals if a complete biotinylation is wished, or if the peptide presents a terminal NH2 alone (more difficult to biotinylate).
- 4- Purify the peptide by reverse phase or any other suitable technique (ion exchange, gel filtration...)

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* **Rem:** Sulfonated biotinylation agents can be dissolved in water or the right quantity directly added to the protein solution.

Protocole 3 : *in situ* biotinylation of cell's proteins

This protocol is designed to biotinylate blood cells, in particular to label proteins presented outside membranes.

- 1- Wash 4 times the cells with cold ** PBS (NaCl 150mM, Phosphate 20mM, pH7.5). Prepare a suspension at 10^6 - 10^8 cells per ml.
- 2- Prepare a solution of sNHS-SS-biotin at 50mM in distilled water or in anhydrous DMSO.
- 3- Add 1- 100 μ M of sNHS-SS-biotin (the concentration depends on the desired detecting signal, of the cell resistance...)
- 4- Incubate 30min at +4°C or at room temperature. Wash 2 times the cells with cold PBS à +4°C

** operating washes and incubations at 4°C prevents usually cell damages.

The biotinylated cells can be followed up after growth *in vitro*, or survival *in vivo* (determination of the cell's life-span, distribution on organism, localisation of degradation in tissues...). Analysis may includes flow cytometry (quantitation of labelled cells, level of biotinylation), and western-blotting (qualitative analysis of labeled proteins) with labelled streptavidins. After lysis and extraction with detergents, biotinylated membrane components can be purified by affinity with immobilized monomeric avidin (#UP29337A).

Protocole 2 : biotinylation of a solid surface

Fritzsche W., Ermantraut E., Köhler J. M ; Characterization of Biomolecule Immobilization by Scanning Force Microscopy Using a Wet-Masking Technique ; Scanning Vol. 20, 106-109 (1998)

Other Information

The level of biotinylation can be estimated by assay of biotin, after or not, digestion of proteins by the pronase (dosage with HABA , or ELISA inhibition)

For use *in vitro* only, not for diagnostic.

For any information, please contact Uptima, or your local distributor.

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