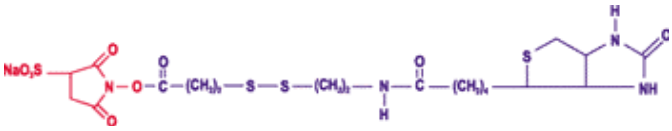
NHS-cleavable-Biotins

Product Description

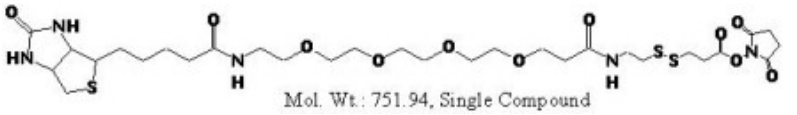
Amine reactive biotinylation reagents, with cleavable spacer (by DTT, DTE, or TCEP r)

Catalog number UPS0738A, 100mg
Nom : **NHS-SS-Biotin**
Succinimidyl-6-(biotinamido)ethyl-1.3-dithiopropionate
Molecular weight: **MW= 504.65** (M)

Catalog number: UP53031A, 100mg UP53031B, 50mg 530318, 8x1mg
Nom : **sNHS-SS-Biotin**
Sulfosuccinimidyl-6-(biotinamido)ethyl-1.3-dithiopropionate
Molecular weight: **MW= 606.7** (M)



Catalog number: CC4431, 50 mg CC4433, 1 g
Name: **NHS-PEO4-SS-Biotin**
NHS-PEO4-SS-Biotin HCl
Molecular weight: **MW= 751.94** (M)



Soluble in Methylene chloride or DMAC and DMSO

Storage : –20°C (M)

Introduction

The **biotin** is a vitamin widely used in biotechnology for its propriety to bind with extremely high affinity to avidin ($Ka=10^{-15}$ M^{-1}) and streptavidin ($Ka=10^{-14}$ 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 it's low MW / volume and hydrophylicity. After a biomolecule has been biotinylated, it is easily detected thanks to labeled (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).

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

Scientific and Technical Information

- The chemical group **N-hydroxysuccinimydyl (NHS)** reacts in aqueous phase on primary (–NH2) and secondary amines (=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 NH2 located in terminal peptidic chains. The reaction accommodates +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 30-300µmol per mg of protein.

- The **sulfonyl moiety (NaSO3)** 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 aqueous buffers, up to 10mM, avoiding the use of organic solvents like DMSO or DMF, that are possibly nocive to cells or applications.

- The **spacer** is available with different lengths, and contains a disulfide 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 10µM of reducing agent at room temperature. Glutathion might be helpful to reduce while maintaining physiological conditions, as it do not penetrate in cells (r), as well as TCEP a membrane impermeant potent and odorless reducer.

  The **lc-lc spacer** of product #UP37924 between biotin and NHS is 24.3Angstroms. This spacer arm favors the availability of Biotin for (strept)avidin, hence finally increases the sensitivity of detection.

  The **PEO spacer** of product #CC443 provides the same length as the lc-lc spacer, and confers hydrophilicity: as a result, the product is soluble in water, confer lowest non-specific binding and minimize aggregation and precipitation after conjugation (a sever problem in some applications with conventional spacers), and is not antigenic nor immunogenic. Hence product CC443 has not only the improved streptavidin binding, but also achieve higher coupling ratio and yields more stable conjugates.

Directions for Use

Main directions for use and scientific information are similar to classic NHS biotins (FT-52117). Modifications of following protocols 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 protein in solution (buffer of biotinylation) up 10mM. Uptima recommends not storing the stock solution, because the product is readily subject to hydrolysis. A limited storage may be obtained when using high quality anhydrous DMSO or DMF under argon or nitrogen gas at –20°C. Using dry DMF is critical. Placing 3 Angstrom or 4 Angstrom molecular sieves into reagent grade DMF, shaking and letting stand at least overnight can easily obtain this. If possible handle the NHS-biotin under an inert atmosphere.

- NHS-PEO4-SS-Biotin is soluble in Methylene Chloride, DMAC, and DMSO.
- The possible conditions of the esterification reaction are various. The biotinylation is usually performed 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 labeling (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 protocol 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, dialyze, or gelfiltrate in the right buffer. Other concentrations can be done, but the coupling ratio should be slightly increased if the antibody is more diluted.

2- Prepare a sNHS-SS-biotin solution at 20mMin distilled water or in anhydrous DMSO.

3- Add 15µL 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.

Protocol 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 lyophilized 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 200-400µl of sNHS-SS-biotin in 1 ml of peptide solution at 5mM
Rem : add up to 4 other times 200µl 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….)

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

Protocol 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- 100µM 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 at +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, localization of degradation in tissues…). Analysis may includes flow cytometry (quantitation of labeled cells, level of biotinylation), and western-blotting (qualitative analysis of labeled proteins) with labeled streptavidins. After lysis and extraction with detergents, biotinylated membrane components can be purified by affinity with immobilized monomeric avidin (#UP29337A).

Protocol 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)

Alternative protocols

Hermanson, Greg T, Bioconjugate Techniques, Academic Press, Inc., San Diego, CA, 1996 (ISBN 0-12-342335-X). Use protocol on page 377, except make a 40 mg/ml solution of the NHS-biotin or NHS-PEO-SS-Biotin in dry DMF.

Other information

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

For use in vitro only, not for diagnostic.

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