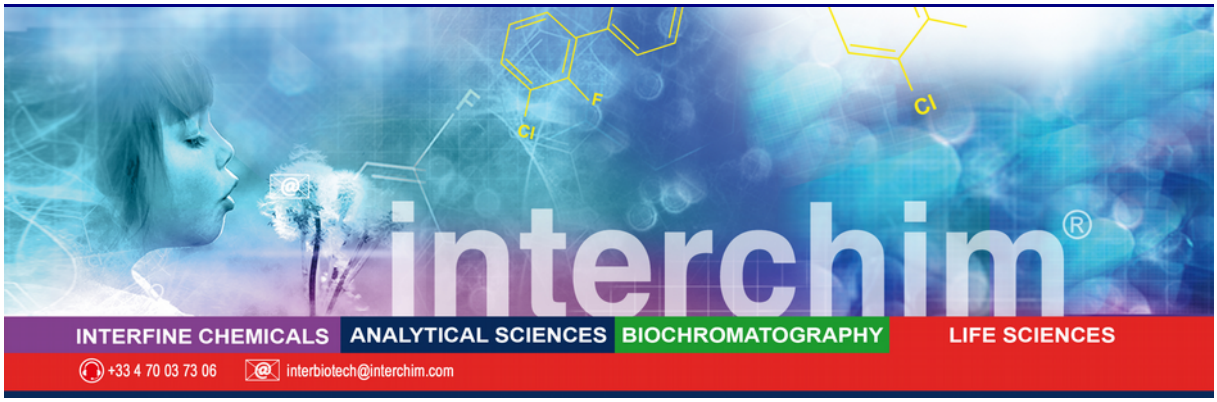
FT-52117A



NHS-x-Biotin, & sulfonamide forms

Products Description

Catalog #:	Name & description
UP39044A, 100mg UP39044B, 50mg	**NHS-Biotin** N-Hydroxysuccinimido-Biotin MW=341.38; Spacer: 13.5A (K)
UP52117A, 100mg UP52117B, 50mg	**sNHS-Biotin** Sulfosuccinimidyl-6-biotin MW=443.42; Spacer: 13.5A (K)
UP85262A, 100mg UP85262B, 50mg	**NHS-lc-Biotin** Succinimidyl-(biotinamido)hexanoate-biotin MW=454.55; Spacer: 22.4A (K) mp 169 °C
UP54398A, 100mg UP54398B, 50mg	**sNHS-lc-Biotin** Sulfosuccinimidyl-6-(biotinamido)hexanoate-biotin MW=556.58; Spacer: 22.4A (K)
UP29847A, 50mg UP29847B, 100mg	**NHS-lc-lc-Biotin** 6-((+)-Biotinamidocaproylamido)caproic acid N-hydroxysuccinimide ester MW=567.71; Spacer: 30.5A (K)
UP37924A, 50mg	**sNHS-lc-lc-Biotin** MW=669.75; Spacer: 30.5A (K)
BT3661, 100mg	**NHS-C10-Biotin** MW=524.68 (K)
Storage :	+4°C (-20°C for long term) (K)

General Considerations

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 molecular weight and steric volume. It is easily detected with labeled (strept)avidins, thus biotin represents a privileged label for antibodies and proteins involved in hapten-ligand interactions.

Interchim offers biotins activated by the succinimidyl ester, easy to use in labs, rendering these 'NHS-biotins' the typical and commonly used "home-made labeling" reagent.

See also NHS-PEOx-Biotins for improved features (hydrophilicity).

Scientific and technical Information

- The chemical group **N-hydroxysuccinimydyl (NHS)** reacts in aqueous phase on primary ($-NH_2$) and secondary amines (=NH) (in fact on its deprotonated form), at pH 7-9.5, and optimally at in a pH ~8.5. The reaction is very specific, targeting typically ε-amines present in Lys amino-acid in any proteins, but in a lower proportion on terminal α-NH_2 of peptide chains. The reaction competes with hydrolysis, which increases with pH and with the high dilutions of the molecule that should be derivatized.

- 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 **hexanoate link (LC= 'long chain')** permits to introduce a spacer arm of 22.4 Angstroms between the labeled molecule and the biotin. LC-LC spacer is 30.5 A long. This increases the availability of biotin to bind to (strept)avidin, and finally increases the sensibility of detection. However, longer spacer do not always give the best result !

Directions for Use

Here are standard protocols, for proteins (1), peptides (2), cells (3). A setting may be needed to optimize the biotinylation level for each protein, the quality of cell label, or any application result. Many other applications (as haptens, nucleic acids) can be found in the Literature.

General guidelines.

- ▪Solubility
  ▪**NHS-Biotins** can be dissolved in DMSO or DMF. Uptima recommends to weight out the needed quantity and not to store a stock solution, because the later may degrade. Using high quality anhydrous DMSO and closing the vial under argon or nitrogen gas at –20°C may reduce product hydrolysis, allowing eventually storage for a limited time).
  ▪**sulfonated Biotinylation** agents (sNHS-Biotin, sNHS-lc-Biotin et sNHS-lc-lc-Biotin) can be dissolved directly in distilled water, up 10mg/ml, but this mother solution should be used immediately. Their main advantage is that the reagent can be added in one step, directly to the protein solution (in buffer of biotinylation), and even as powder form. Alternatively, see NHS-PEOx-Biotins.
- The possible conditions of the esterification reaction are various.
  ▪**Buffer**▪ The biotinylation is usually performed in a neutral buffer, like PBS (NaCl 150mM, phosphate 20mM, pH7.4), or carbonate or others (but not in Tris or glycine buffers).
  ▪**Ratio**▪ Use 1-50 reactive biotin per amine or protein to label. Typically, a ratio of 3-10 biotin to protein molecule is used to achieve 1-3 coupled biotin per protein; and 10-20 ratio are used for strong biotinylation of large proteins.
  ▪**Incubation**▪ Incubation may operate for 30min to 4H depending on temperature (Room Temperature usually, or +4°C for labile proteins or cells).

- **▪Desalting▪**
  It is usually necessary to remove by-products after labeling (excess of NHS-biotin, free biotin and NHS).

Protocol 1 : biotinylation of an antibody (protein)

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 protein (antibody) at 5mg/ml in PBS (NaCl 150mM, Phosphate 20mM, pH7.5).

This can be done by dissolving the lyophilized protein, or by dilution. Check that the protein does not contain other proteins or Tris or other interfering agents. Else, purify, dialyze, or gelfiltrate in the right buffer. Refer also to general guidelines.
1-10mg/ml protein concentrations can be used, but the coupling ratio should be increased if the antibody is more diluted.

2- Add the biotinylation agent to the protein solution at the desired ratio (10-20 fold molar excess)

For instance -Non-sulfonated biotins (i.e. NHS-Biotin):
Prepare a 20mM solution in anhydrous DMSO.
Add 15µL of the solution of NHS-biotin to the antibody (1ml-5mg).
-Sulfonated biotins:
Weight 18.5mg of sNHS-lc-Biotin ° and add it to the protein solution (1ml-5mg) under agitation.

3- Incubate 1H at room temperature.

4- Dialyze the antibody against PBS+NaN_3 0.01% (Use CelluSep membranes). The biotinylated antibody can be diluted to 1mg/ml at 0.1% NaN3 and 20% of glycerol for storage at–20°C or +4°C.

The level of biotinylation is in the range of 1-3 biotins per IgG. This can be estimated by quantification of biotins by the HABA method (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:

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 NHS-biotin at 50mM in anhydrous DMSO *.

3- Add 200-400µl of NHS-biotin in 1 ml of peptide solution at 5mM. Incubate for 30-60min.
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.

- Use sulfonated biotin to biotinylate proteins presented outside membranes
- Use non sulfonated biotin to biotinylate intracellular proteins

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 NHS-biotin at 50mM in anhydrous DMSO *

3- Add 1- 100µM of sNHS-biotin (the concentration depends on the desired detecting signal, the cell resistance,…)

4- Incubate 30min at +4°C** or at room temperature. Wash 2 times the cells with cold PBS** at +4°C

* sulfonated biotinylation agents can be dissolved in water or the right quantity directly added to the cell suspension.

** 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) using a labeled streptavidin. After lysis and extraction with detergents, biotinylated membrane components can be purified by affinity with monomeric avidin (#UP29337A).

Other information

For use in vitro only, not for diagnostic.

The level of biotinylation can be estimated by assay of biotin, after or no digestion of proteins by the pronase enzyme (dosage with HABA , or ELISA inhibition)

Literature:

Biotinylation of proteins

"Optimizing antibody immobilization strategies for the construction of protein microarrays." Peluso P, Wilson DS, Do D, Tran H, Venkatasubbaiah M, Quincy D, Heidecker B, Poindexter K, Tolani N, Phelan M, Witte K, Jung LS, Wagner P, Nock S. Anal Biochem 312, 113-24 (2003)

· "In-situ monitoring of protein labeling reactions by matrix-assisted laser desorption/ionization mass spectrometry." Lu J, Zenobi R. Fresenius J Anal Chem 366, 3-9 (2000)

· "Peptide biotinylation with amine-reactive esters: differential side chain reactivity." Miller BT, Collins TJ, Rogers ME, Kurosky A. Peptides 18, 1585-1595 (1997)

Edberg, J. C., and Kimberly, R. P. (1992) *J. Immunol. Methods* **148,**179-187

Biotinylation of peptides / haptens

· "Radiolabeled biotinyl peptides as useful reagents for the study of proteolytic enzymes." Basak A, Boudreault A, Jean F, Chretien M, Lazure C. Anal Biochem 209, 306-314 (1993)

· "Biotinylated human beta-endorphins as probes for the opioid receptor." Hochhaus G, Gibson BW, Sadee W. J Biol Chem 263, 92-97 (1988)

· "Preparation of fluorescent, cross-linking, and biotinylated calmodulin derivatives and their use in studies of calmodulin-activated phosphodiesterase and protein phosphatase." Kincaid RL, Billingsley ML, Vaughan M. Methods Enzymol 159, 605-626 (1988)

Biotinylation of cells

Miyake, K., Weissman, I. L., Greenberger, J. S., and Kincade, P. W. (1991) *J. Exp. Med.***173,** 599-607

"Metabolic behavior of cell surface biotinylated proteins." Hare JF, Lee E. Biochemistry 28, 574-580 (1989)

Biotinylation of amino-bearing nucleotides

"Preparation of fluorescent, cross-linking, and biotinylated calmodulin derivatives and their use in studies of calmodulin-activated phosphodiesterase and protein phosphatase." Kincaid RL, Billingsley ML, Vaughan M. Methods Enzymol 159, 605-626 (1988)

"Synthesis and hybridization of a series of biotinylated oligonucleotides." Cook AF, Vuocolo E, Brakel CL. Nucleic Acids Res 16, 4077-4095 (1988)

"*In Vitro* Synthesis of Biotinylated RNA Probes from A-T Rich Templates: Problems and Solutions." Luehrsen KR, Baum MP. Biotechniques 5, 660 (1987)

"Novel chemical method for the preparation of nucleic acids for non-isotopic hybridization." Viscidi RP, Connelly CJ, Yolken RH. J Clin Microbiol 23, 311-317 (1986)

Related product and documents:

See BioSciences Innovations catalog and e-search tool.
▪Other biotins/ Including NHS-PEOx-Biotins for improved features
ex: UPR2027A has same reactivity than UP379A2, similar spacer length, similar water solubility, but confers hydrophilicity to the conjugate for improved features: higher solubility, less aggregation, lower background, ...)
▪HABA #UP05361D (for quantitation of Biotin)
▪Desalting tools, i.e. CelluSep dialysis tubings

For any information, please contact Uptima, or your local distributor.
213 av. J.F.kennedy, 03103 Montlucon, fax : +33(0)4 70 03 82 60, hotline Interbiotech : +33(0)4 70 03 73 06

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