

NHS-x-Biotin, & sulfonated forms

Product Description

Catalog Number:	UP39044A, 100mg	UP39044B, 50mg
Name :	N-Hydroxysuccinimido-Biotin (NHS-Biotin)	
Formula :	$C_4H_4O_2N-O-CO-(CH_2)_4-C_5SN_2OH_7$, M.W.=341.38	
Catalog Number:	UP52117A, 100mg	UP52117B, 50mg
Name :	Sulfosuccinimidyl-6-biotin (sNHS-Biotin)	
Formula :	$NaO_3S-C_4H_3O_2N-O-CO-(CH_2)_4-C_5SN_2OH_7$, M.W.=443.42	
Catalog Number:	UP85262A, 100mg	UP85262B, 50mg
Name :	Succinimidyl- (biotinamido)hexanoate-biotin (NHS-1c-Biotin)	
Formula :	$C_4H_4O_2N-O-CO-(CH_2)_5NH-CO-(CH_2)_4-C_5SN_2OH_7$, M.W.=455.55	
Catalog Number:	UP54398A, 100mg	UP54398B, 50mg
Name :	Sulfosuccinimidyl-6-(biotinamido)hexanoate-biotin (sNHS-1c-Biotin)	
Formula :	$NaO_3S-C_4H_3O_2N-O-CO-(CH_2)_5NH-CO-(CH_2)_4-C_5SN_2OH_7$, M.W.=556.58	
Catalog Number:	UP29847A, 50mg	UP29847B, 100mg
Name :	6-(+)-Biotinamidocaproylamido)caproic acid N-hydroxysuccinimide ester (NHS-1c-1c-Biotin)	
Formula :	$C_{26}H_{41}N_5SO_7$, M.W.=657.7	
Catalog Number:	UP37924A, 50mg	
Name :	(sNHS-1c-1c-Biotin)	
Formula :	$C_{26}H_{40}N_5S_2O_{10}$, M.W.=669.75	
Storage :	-20°C (possible at +4°C) (M)	

General Considerations

The biotin is a vitamin widely used in biotechnology for its propriety to bind with extremely high affinity to avidin ($K_a=10^{15} M^{-1}$) and streptavidin ($K_a=10^{14} M^{-1}$). 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 its low molecular weight and steric volume. It is easily detected with labelled (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 labelling" reagent.

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), 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 competes with hydrolysis, that increases with pH, and with the high dilutions of the molecule that should be biotinylated.
- 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 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 labelled 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 !

Use

Here are some protocols. A setting may be needed to optimize the biotinylation level for each protein, the quality of cell label, or any application result.

- **NHS-Biotins** can be dissolved in DMSO. 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 gaz at $-20^\circ C$.
Biotinylated sulfonated agents (sNHS-Biotin, sNHS-Ic-Biotin et sNHS-Ic-Ic-Biotin) can be dissolved directly in distilled water (this solution should be used immediately), or added directly to the proteic solution (buffer of biotinylation).
- 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 (but not in Tris buffers).
- It is usually necessary to remove by-products after labelling (excess of NHS-biotin, free biotin and NHS).

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 NHS-biotin solution at 20mM in anhydrous DMSO.
 - 3- Add 15 μ L of the solution of NHS-biotin to the antibody (1ml).
* Rem: Sulfonated biotinylation agents can be dissolved in water or the right quantity directly added to the protein solution.
- Incuber 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 $-20^\circ C$ or $+4^\circ C$.

The level of biotinylation is in the range of 1-3 biotins per IgG. This 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:

- 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 NHS-biotin at 50mM in anhydrous DMSO *.
- 3- Add 200-400µl of NHS-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.

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

This protocol is designed to biotinylate blood cells.

- Use sulfoned biotin to label proteins presented outside membranes
 - Use non sulfoned biotin to intrinsic label
- 1- Wash 4 times the cells with cold ** PBS (NaCl 150mM, Phosphate 20mM, pH7.5). Prepare a suspension at 10⁶-10⁸ 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, 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

* 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, 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 monomeric avidin (#UP29337A).

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.

Related product and documents:

PLP.Biotins

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

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