

Hydrazide- Biotin

Oses-reactive activated biotins

Description

Catalog number:	UP78631A, 100mg	UP78631B, 50mg
Name :	Hydrazide-Ic-Biotin	
Formula :	Biotin-ε-aminocaproyl hydrazide, C ₁₆ H ₂₉ N ₅ O ₃ S, CAS(109276-34-8), M.W.= 371.5	
Cat.number:	UP36466A, 100mg	UP36466B, 50mg
Name :	Hydrazide-Biotin	
Formula :	Biotinyl-hydrazide, C ₁₀ H ₁₈ N ₄ SO ₂ ; CAS(66640-86-6), M.W.= 258.3	
Storage :	+4°C protect from light and moisture R: 23/24/25,36/37/38, S: 45,26,36,22	

General Considerations

The biotin is a vitamin widely used in biotechnology for its propriety of binding with extremely high affinity to avidin ($K_a=10^{15} M^{-1}$) and streptavidin ($K_a=10^{14} M^{-1}$). This hapten-protein interaction resists effectively to drastic physico-chemical conditions, allowing various immuno-technologies, and notably detections. The biotin can be conjugated through several chemical reactions with molecules of interest. Besides the common attachment to amines (that operates on numerous sites on the molecule, and generally doesn't affect the biological activity), attachment through carbohydrate allows original applications. The labeling also applies too to glycosylated native proteins, but a reducing step is needed, except when reduced osidic groups are available. For example immunoglobulins can be biotinylated without interfering with the specific activity, and with better stereoscopic availability to detect agents. Hydrazide biotin also allows the labeling of sugars, glycolipids, and nucleic acids. As it is easily detected by labeled (strept)avidins, biotin represents a privileged tool for the labeling of probes (detection purposes), and proteins studies (structure elucidation, hapten-ligand interactions).

Interchim offers aldehyde reactive biotin derivatives to answer the needs in many detection systems and protein research applications (other reactive biotins are available): hydrazide-Biotins have been used to label **glycoproteins** (Wilchek 1987), **glycolipids**, **sialic acids and sugars** (Baver 1988) **steroids** (Tiefenauer 1990), **LDL** (Wade 1988), and **nucleic acids** (Arawawa 1989, Arawal 1986), but also N-terminal serine and threonine residues in proteins.

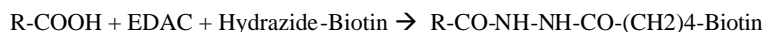
- **Protein studies**: study of the interaction between biomolecules and of complexes (biotinylated ligands/receptors) (Yamamoto 1984); elucidation of the structure of proteins after labeling beared glycones; labeling of complexe mixture to identify glycosylated molecular species (by immunoblotting or suitable technique)...
- Obtention of **labeled affine probes**: for example, biotinylated antibodies on Fc through their glycone, biotinylated haptens (drug, hormone...) to use as a tracer in ELISA, identification of a receptor after interaction with its biotinylated ligand.....
- **Purification** of biotinylated molecules, or molecules having bound to a biotinylated ligand: a biotinylated molecule, or its complexe, can be affinity purified from a complex mixture (detergent cell extract) with an immobilized avidin support (#UP34090A and related products)

Scientific and Technical Information

- Hydrazide biotin is soluble up to 20mg/ml in DMSO.
- The hydrazide group reacts specifically with aldehyde groups, forming a stable hydrazone bond.



- Hydrazide also reacts with carboxylic acids in the presence of EDAC (#UP520050)



EDC reaction with COOH is usually performed in an acidic buffer (pH 4.7-5.5), but coupling can actually be accomplished in a buffer system up to pH 7.4. one must keep in mind that some proteins have both carboxylic acids and primary amines available. In this

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situation, in addition to biotinylating the molecule using the EDC, polymerization of the molecule is possible. This can be minimized by decreasing the amount of EDC used and/or increasing the amount of Biotin Hydrazide used. Alternatively, the amines on the molecule to be biotinylated can be blocked using Sulfo -NHS-Acetate (UP69380).

Use

These standard protocols are given as an example, and should be optimized for each protein and application.

Protocol 1: Biotinylation a CHO-bearing molecules with Hydrazide-Biotin

Protein generally does not contain free aldehyde; this group can be generated from sugars by mild oxidation with periodate. The protocol is designed for immunoglobulins, but should be applied to any glycosylated protein.

- 1- Prepare a solution of meta-periodate at 20mM in 0.1M sodium acetate buffer pH5.5
This solution should be kept in the dark at 0-4°C, and used immediately. Throw away after use.
- 2- Prepare the protein solution at 5mg/ml in cold 0.1M sodium acetate buffer pH5.5
The protein concentration can be determined by the Bicinchoninic method (#UP40840A, BC Assay).
- 3- Add 1 ml of periodate solution to 1 ml of protein solution. Mix and incubate for 5min at 0-4°C
Remark: the ratio and incubation time should be optimized depending on the protein nature and concentration.
- 4- Dessalt the biotinylated protein by dialysis or gel filtration in 0.1M sodium acetate buffer pH5.5
Fractions containing the biotinylated protein can be identified by measuring the absorbance at 280nm, or any other means. Pool interesting fractions.
- 5- Prepare a Hydrazide-Biotin solution at 40mM in DMSO.
- 6- Add 250µl of Hydrazide-Biotin solution to 2 ml of protein solution. Mix and incubate for 2H at room temperature.
- 7- Dessalt the biotinylated protein by dialysis or gel filtration with PBS (NaCl 150mM, phosphate 10mM pH7.4).
Fractions containing the biotinylated protein can be identified by measuring the absorbance at 280nm, or any other means and analysed. Pool interesting fractions.
- 8- Biotinylated antibodies can be stored in PBS + 0.1% NaN₃ and 50% glycerol at -20°C.

Remark: The oxidation can be performed by other technics (galactosidase oxidase, neuraminidase...).

Protocol 2: Biotinylation a COOH-bearing molecules with Hydrazide-Biotin

- 1- Prepare the protein solution at 5mg/ml in 0.1M MES (2-N-morpholino-ethanesulfonic acid) pH5.5
- 2- Prepare a 50mM solution of Hydrazide biotin in DMSO
- 3- Add 25µl of biotin-hydrazide to 1ml of protein solution. Mix.
- 4- Prepare a 10mg/ml solution of EDAC (#UP52005) in 0.1M MES pH5.5. Use immediately
- 5- Add 12.5µl of the EDC solution. Mix and incubate overnight at room temperature under constant agitation.
- 6- Dessalt the biotinylated protein by dialysis or gel filtration with PBS (NaCl 150mM, phosphate 10mM pH7.4). Fractions containing the biotinylated protein can be identified by measuring the absorbance at 280nm, or any other means and analysed. Pool interesting fractions.

Other Information

Literature

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

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