

FT-78631A

Hydrazide- Biotin Carbohyhydrate-reactive activated biotins

Hydrazide biotins are biotinylation reagents for labeling carbohydrates and glycoproteins or glycolipides via their glycone that are oxidable. Notably useful for labeling antibodies with improved activity.



Products Description

Technical information

Biotin label

The **biotin** is a vitamin widely used in biotechnology for its propriety of binding with extremely high affinity to avidin (Ka= 10^{-15} M⁻¹) and streptavidin (Ka= 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 chemically with biomolecules of interest, though several groups. This sheet describe those activated with hydrazide, and available with different spacer lengths.

The **hydrazide** (—NH-NH2) functional group reacts with aldehydes, Carboxylic acide and ketones. It provides a interesting and simple method to conjugate glycoproteins and other carbohydrate-containing compounds having oxidizable sugars or aldehydes.



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Hydrazides react specifically with aldehyde groups in slightly acidic conditions to form hydrazone linkages; these can be further reduced to stable secondary amine bonds using sodium cyanoborohydride (Part N°057771). The reaction is more efficient in the presence of aniline (Part No. 88944). Alternatively, hydrazides can be conjugated to carboxylic acids using EDC carbodiimide chemistry.

Hydrazide reacts with aldehydes formed by periodate-oxidation of sugar groups, yielding a semi-permanent hydrazone bond. Perform reaction at pH4 to 6 in buffers such as sodium acetate.

Spacer

3 spacer lengths (see description) are available. Longer spacer reduces potential steric hindrance of biotin and conjugated molecule. The PEO₄ spacer (polyethylene oxide, or PEG) is additionally hydrophilic and imparts several benefits:

- water solubility : the reagent can be dissolved directly in aqueous solutions
- the hydrophilicity is transferred to the biotinylated molecule (not as with sulfoNHS-Biotins), hence :
- \bullet you can achieve higher coupled ratios

• you reduce eventual aggregation and precipitation of labeled proteins, that commonly occur when labeling antibodies and other biological materials. This minimize artifacts or background in detections, and protein loss during storage

- \bullet you reduce non-specific binding on surfaces
- \bullet no immunogenicity is conferred to the conjugate

Solubility

- all Hydrazide biotin are soluble up to 20mg/ml in DMSO. Solubility is lower in DMF. For direct solubilisation in aqueous solutions, NHS-PEO₄-Biotin is recommended (up 20mM), even it is possible also with products #UP36466 and #UP78631 (only at < ca 5mM).
- The spacer arm of Biotin-PEO₄-Hydrazide #BJ008 is hydrophilic, hence it eliminates or minimizes non-specific binding that causes aggregation and precipitation problems, which commonly occur when labeling antibodies and other biological materials. Additionally, PEO (also known as dPEG) spacer is non-immunogenic.

Coupling group reactions

The **hydrazide group** is a useful coupling group, allowing conjugation to aldehydes, and (upon EDC mediated activation) to carboxyls. It provides thus a privileged method to conjugate a variety of biomolecules: Hydrazide-Biotins have been used to label **glycoproteins**(<u>Wilchek 1987</u>), **glycolipids**, **sialic acids and sugars** (<u>Bayer 1988</u>) **steroids** (<u>Tiefenauer 1990</u>), **LDL** (<u>Wade 1988</u>), **and nucleic acids** (<u>Arakawa 1989</u>, <u>Agrawal 1986</u>), but also N-terminal serine and threonine residues in proteins.

.For reducing sugars (containing free CHO groups), direct conjugation is possible, but most other applications require a reducing or an oxidizing step to generate CHO groups from carboxyls or from cis-diols. See below 'Coupling carbohydrates or glycoproteins'.

.Lastly, hydrazide allows for useful conjugation of **peptides**/proteins through their carboxyl groups in specific applications (*oriented conjugations*). See below 'coupling carboxyls".

Coupling CHO groups (carbohydrates or glycoproteins)

- Aldehyde group have first to be generated if not already present on the molecule to biotinylate. This can be achieved by: -Sialic acids is easily oxidized with 1 mM sodium periodate (NaIO₄).
 -reducing oses can be oxidized effectively with 5-10 mM sodium periodate. This applies to glycoproteins, to create CHO groups.
 -hydrazide can alternatively by graffted chemically using a crosslinker HYD-NHS such as SATH #<u>UP52005A</u>, HYD-MAL such as MBPH #<u>09835A</u> or EMCH #<u>UPG991A</u>.
- The hydrazide group reacts specifically with aldehyde and ketone groups, forming a stable hydrazone bond in a single step.

R-CHO + Hydrazide-Biotin \rightarrow R-CH=N-NH-CO-(CH₂)₄-Biotin

.Compared with conventional labeling through amines (ubiquitous in proteins), the attachment through aldehydes (present on or generated on carbohydrates) is a useful approach for glycoproteins such as antibodies, and glycolipids. **Biotinylation via sugar moieties of antibodies** typically provides the best orientation for the biotin label or conjugated molecule (better stereoscopic availability for (strept)avidins detection or capture reagents), as the sugar groups are associated with the Fc region of the antibody, while leaving the antibody active sites and light chains free to bind their target (better ab reactivity). The method however require cis diols of the sugars first be oxidized to aldehyde groups, which can then react with hydrazide-biotin (<u>alt.</u>). In few cases this can impair the stability or reactivity of very fragile antibodies (notably monoclonals). Furthermore, monoclonal antibodies may be deficient in glycosylation. All that makes useful to validate the method also for any application, and including with other protein types.

Coupling COOH groups (carboxyls)

 Hydrazide reacts



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with carboxyl groups in the presence of EDAC (#UP52005A):

R-COOH + EDAC + Hydrazide-Biotin \rightarrow R-CO-NH-NH-CO-(CH₂)₄-Biotin

This occurs with aspartate and glutamate residues or on the carboxy terminus of proteins, and carboxy group of reducing end of polysaccharides (oxidize sugar groups using either a specific oxidase (i.e. galactose oxidase), or 1-10 mM sodium meta-periodate (NaIO4). Oxidation with periodate is most efficient in acidic conditions (i.e. 0.1 M sodium acetate, pH 5.5), although neutral buffers such as phosphate buffered saline can be used. If oxidation is performed in acidic conditions, buffer exchange by dialysis or gel filtration into neutral buffer may be necessary to obtain optimal hydrazide reaction

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. Use MES buffer for example; phosphate buffers can be used but reduce conjugation efficiency, although this effect can be overcome by adding more EDC. Avoid using buffers like Tris, Glycine, acetate, citrate,...! The activated biotin reacts with hydrazide, yielding the right conjugate, but also with amines; Thus in most cases with proteins (that have both carboxylic acids and primary amines available) a polymerization of the molecule is possible. This can be minimized by decreasing the amount of EDC used and/or increasing the amount of used Biotin Hydrazide . Alternatively, the amines on the molecule to be biotinylated can be blocked using Sulfo-NHS-Acetate (UP69380).

Applications

- Preparation of labeled probes: especially, antibodies biotinylated through their glycone on Fc fragment preserving antibody recognition site, biotinylated haptens (drug, hormone...) to use as a tracer in ELISA, lipopolysaccharides ^(Luk 1995), biotinylated hyaluronan ^(Yu 1995),...
- Cell surface labeling of glycoproteins, i.e. leukocyte surface proteins (Kahne 1994)
- Affinity Purification: a biotinylated molecule (peptide), or its complex with its ligands (receptor), can be affinity purified from a complex mixture (detergent cell extract) with an immobilized avidin support (#UP34090A and related products); Such affinity method provides a powerful pull down assay to identify a receptor after interaction with its biotinylated ligand.
- **Protein studies**: study of the interaction between biomolecules and complexes (biotinylated ligands/receptors) (Yamamoto 1984); elucidation of the structure of proteins after labeling their glycones; labeling of complex mixture to identify glycated molecular species by suitable technique (analysis-based: immunoblotting, or separation-based: chromatography)

Directions for Use

The following standard protocols are given as examples, and should be optimized for each protein and application. Please refer to the literature. Especially, <u>Greg Hermanson manual</u> gives the protocol for the coupling of the hydrazide to an oxidized glycoprotein.(applications pages 390-393). Note too that this can be carried out without the reduction with NaCNBH₃ as well. However, the reduced forms are stable to a wider pH range.

Avoid Tris or other primary amine-containing buffers in the oxidation and biotinylation steps as these buffers react with aldehydes and will quench the reaction with hydrazides.

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

Proteins generally do not contain free aldehyde; this group can be generated from sugars (or carbohydrates of glycoproteins) by mild oxidation with periodate. (note: oxidation can be performed by other techniques, i.e. galactosidase oxidase, neuraminidase...). The protocol is designed for immunoglobulins, but should be applied to any glycated protein (i.e. see ^{D/Angene 2006}).

- 1- Prepare a solution of meta-periodate (NaIO4) 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 Acid 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- Desalt the protein by dialysis or gelfiltration 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 mean, and pooled.
- 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 gelfiltration with PBS (NaCl 150mM, phosphate 10mM pH7.4). Fractions containing the biotinylated protein can be identified by BC Assay #UP40840A, or any other means and pooled.



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8- Biotinylated antibodies can be stored in PBS + 0.1% NaN3 and 50% glycerol at -20° C.

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

- 1- Prepare the protein solution at 5mg/ml in 0.1M MES (2-N-morpholino-ethanesulfonic acid) pH5.5
- 2- Prepare a 50mM solution fo Hydrazide biotin in DMSO
- 3- Add 25µl of biotin-hydrazide to 1ml of protein solution. Mix.
- 4- Prepare a 10mg/ml solution of EDAC (#UP52005A) 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- Desalt the biotinylated protein by dialysis or gelfiltration with PBS (NaCl 150mM, phosphate 10mM pH7.4). Fractions containing the biotinylated protein can be identified by BC Assay #UP40840A, or any other means and pooled.

Literature

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Other information

Related products and documents

Sodium Cyanoborohydride #057771NHS-PEOx-Biotins FT-R2027AHydrazide-Agarose #AWJK30Sulfo-NHS-Acetate #UP69380Maleimido-Biotins FT-BU9730MAL-PEOx-NHS FT-AL6581BC Assay protein dosage FT-40840AEDAC FT-52005Aavidin support #UP34090ADesalting tools (CelluSep dialysis tubings; desalting columns)Hydrazone chemistry: Conjugation kit #BL1501and crosslinkers (SANH #BL9270, MHPH #BL9401)

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