FT-48198A

Thiol reactive biotins

Maleimido-, HPDP-, Iodoacetyl- activated Biotins

Products Description

Catalog #:	Name & description
481981, 100mg	**Maleimido-Biotin** $C_{20}H_{29}N_5O_5S$, **M.W.= 451.5** (L) N-Biotinoyl-N′-(6-maleimidohexanoyl)hydrazide
996874, 50mg	**Maleimido-lc-Biocytin** N-Biotinyl-N-(3-MaleimidoPropionyl)-L-Lysine $C_{23}H_{33}N_5O_7S$, CAS : 102849-12-7, **M.W.= 523.6** (L) increased spacer
UP27443A, 100mg UP27443B, 50mg	**BMCC-Biotin** $C_{26}H_{39}N_5O_5S$, **M.W.= 533.7** (L) (1-Biotinamido)-4[4'-(maleimidomethyl)-cyclohexanecarboxamido]hexane 32.6 A long spacer
UP83035A, 100mg UP85035B, 50mg	**HPDP-Biotin** N-(6-(Biotininamido)Hexyl)-3'-(2'-Pyridylthio)Propionate **M.W.= 539.8** (L) S-S bond allow the spacer to be cleaved by reducing agents
UP55533A, 100mg UP55533B, 50mg	**Iodoacetyl-Biotin** N-IodoAcetyl-N-biotinylhexylenediamine **MW = 510.4** (L)
	Other thiols reactive Biotins:, with a PEO spacer See technical sheet FT-R2028A Biotin-PEO3-iodoacetamide #UP88365A Maleimido-dpEO2-Biotin #BT3750 Maleimido-Biotin PEO2 #UP87284A, PEO3' #UPR2028A and PEO11 #BR4032.

Storage : +4°C protect from light and moisture (desiccate). Possible at –20°C for long term.(L)

Introduction

The biotin is a vitamin widely used in biotechnology for it's 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, and notably detections. The biotin can be conjugated through several chemical reactions to molecules of interest. Besides the common biotinylation though amines, biotinylation through sulfhydryls allows unique applications. The labeling can be performed on native proteins, when free sulfhydryls are available outside, or often preferably to sulfhydryls that have been introduced in proteins or other biomolecules. As it is easily detected by labeled (strept)avidins, biotin represents a privileged tool for labeling probes (detection purposes), and proteins studies (structure elucidation, hapten-ligand interactions).

Labeling native sulfhydryls may be useful for function/structure studies, but avoided for detection purposes, because that can affect the biological activity of the molecule (i.e. the active site includes sulfhydryls). Also, the labeling can be applied to fragments of polymeric proteins, from which the –S-S- bonds, responsible of the tertiary and quaternary structure, have been broken to generate free sulfhydryls. Finally, sulfhydryl can be introduced chemically using SATA reagent (UP84235A) [in proteins or any biomolecule], during synthesis of peptides, or by genetic engineering methods (cys residues). The later methods offer site-defined modification of labeling (SCAM).

Interchim offers sulfhydryl reactive biotin derivatives for the targeting of sulfhydryls, to answer the needs of coupling proteins and peptides in many detection systems and protein research applications (other reactive biotins are available):

- **Protein studies**: study of the interaction between biomolecules and of complexes (biotinylated ligands/receptors) (Yamamoto 1984) ; elucidation of the structure of proteins after labeling a Cys containing regions (Green 1971) ; labeling of complex mixture to identify free SH molecular species (by immunoblotting or suitable technique); analysis of enzymes containing a cys in their active site…
- Preparation of **labeled affine probes**: for example, biotinylated fragments (Fab'2, Fab' and FcSv) of antibodies for the detection on cells, biotinylated haptens (drug, hormone…) to use as a tracer in ELISA, identification of a receptor after interaction with it's biotinylated ligand…
- Preparation of **biologically active conjugates**: specific IgG coupled to drugs for immunotargeting techniques, immunotoxins, …
- **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. (Ghebrehiwet 1979).

Scientific and technical Information

- **The maleimide group of Maleimido-Biotins** reacts very specifically with sulfhydryls –SH at neutral pH 6.5-7.5. The reaction is rapid (few minutes for cystein), and it is well stable. The competitive hydrolysis forming maleamic acid becomes noticeable when pH go up 8.0, where the reactivity with amines begins to be possible. In usual conditions, one should start with a ratio of 10-20 moles of maleimide per mole of protein. With SH-peptides, a molar 1:1 incubation ratio allows almost 1:1 coupling.

- **The pyridyl thiol group of HPDP** reacts specifically at pH7-9 by exchange with sulfhydryl, leaving a pyridin-2-thione group that can be followed up: maximum absorption occurs at 343nm with an extinction coefficient of $8.03 .10^3$ M^{-1} cm^{-1} (Struchbury 1975). The formed link icludes a –S-S- bound; this spacer arm of HPDP measures 29.2 Angstroms length. It increases the availability for ligands binding (streptavidin, avidin). Furthermore, although stable under physiological conditions, it is easily cleavable by thiols using 50mM DTT (#UP28425). The original protein is restored, without modifications as encountered with some other reversible biotinylation reagents.

- **Iodoacetyl group of Iodoacetyl-Biotin** reacts rapidly with thiols by nucleophilic substitution at pH7.5-8.5 giving a thioether bond. At lower or higher pH, with high ratios and long incubation times, undesired reaction may occur, for example with amines and histidines (Gurd 1967) . The extended spacer of 27.1 Angstroms allows high sensitivity of detection, as for HPDP, but it is non cleavable.

- **Spacers** characteristics can modulate avidin/streptavidin binding, as well the interaction of the conjugate with other partner molecules. The products described in this technical sheet have variable spacer length and type (acyl, cyclohexane). The spacer of HPDH contains a disulfide bridge that can be cleaved by reduction, allowing unique downstream applications (i.e. release of the ligand that was biotinylated, captured by an avidin support).
  Ask for new reagents having a PEO structure spacer (see technical sheet FT-R2028A): their hydrophilic spacer confers several benefits over conventional spacers: they eliminates or minimizes non-specific binding that causes aggregation and precipitation problems, which commonly occur when labeling antibodies and other biological materials. Several lengths are available, from 24.9 Angstrom (21 atoms) /PEO2 #87284A to 50.5 Angstrom /PEO11 #BR4032.
  -Additionally, PEO spacer are non-immunogenic.

- **Solubility** in water is usually good up 20mM (UP48198: 20mg/ml in acetic acid). For higher solubility, see Maleimide-PEO-Biotins.

Directions for Use

Following standard protocols are given as examples, and should be optimized for each protein and application.

Protocol 1: Biotinylation a SH-bearing protein with HPDP-biotin

If the protein does not contain free sulfhydryls, this group can be introduced with Traut's reagent (#UP42425), or generated by reduction with DTT, TCEP, or mercaptoethanolamine.

1- Prepare a solution of HPDP at 4mg/ml in DMF or DMSO.
2- Prepare the protein solution at 1-5mg/ml in PBS (NaCl 150mM, phosphate 20mM pH7.5), 4mM EDTA

The protein concentration can be determined by the Bicinchoninic method (#UP40840, BC Assay)

3- Add 2-20µl of HPDP solution per mg of protein to label. Mix and Incubate for 2H at room temperature or 1H at 37°C.

Rem: the exact ratio of HPDP to protein should be determined depending on the protein nature (SH content) and concentration.

Rem: the reaction can be monitored by measuring the absorbance at 343nm.

The incubation duration may be shortened or increased for optimal results in some applications.

4- Desalt the biotinylated protein by dialysis or gelfiltration in PBS. Fractions containing the biotinylated protein can be identified by measuring the absorbance at 280nm, or any other mean. Interesting fraction can be analysed separately, or pooled for further use.

Protocol 2: Biotinylation a SH-bearing peptide with Maleimido-biotin

A cysteine residue can be incorporated at the N-terminus of peptides during the synthesis. If the peptide does not contain free sulfhydryls, this group can be introduced with Traut's reagent (#UP42425).

1- Prepare a solution of Maleimido-Biotin at 40mM (18mg/ml) in DMF or DMSO.

2- Prepare the peptide solution at 10mM in PBS (NaCl 150mM, phosphate 20mM pH7.5)

Lyophilised peptides can simply dissolved in PBS provided there is no preservatives. Proteins in solution can be dialysed or gelfiltrated.

3- Add 250µl of Maleimido-Biotin solution per ml of peptide to label. Mix and Incubate for 30min at room temperature.

The incubation duration may be shortened or increased for optimal results in some applications.

4- Desalt the biotinylated peptide by reverse-phase, gelfiltration or any suitable technique.

Protocol 3: Biotinylation a reduced IgG with Iodoacetyl-biotin

Labeling IgG throught SH avoids the inactivation that occurs when labeling some antibodies thought their amines. IgG does not contain free sulfhydryls available for biotinylation. But SH can be generated by reduction with mercaptoethylamine without impairing the IgG biological activity, and protected by the presence of EDTA (Yoshitake 1979) .

1- Prepare an IgG solution at 10mg/ml (or more) in 0.1M sodium phosphate 4mM EDTA pH6.0 (or dialyse)

2- Add 3.2mg of mercaptoethylamine.HCl to 0.2ml of IgG solution. Mix and Incubate at 37°C for 2H.

3- Gelfiltrate the reduced IgG with cold buffer (Carbonate 100mM, EDTA 4mM, pH8.3). The IgG should be recovered with a concentration of 2mg/ml; or above allowing better reaction yields

4- Prepare a solution of Iodoacetyl-Biotin at 2mg/ml in DMF or DMSO (or in aqueous buffer)

5- Add 10µl of Iodoacetyl solution per ml of reduced IgG (2mg/ml). Mix and Incubate for 2 hours, protected from light at room temperature.

6- Dialyse or gelfiltrate in appropriate buffer.

Literature:

Bayer E; et al; 3-(N-Maleimido-propionyl)biocytin: a versatile thiol-specific biotinylation reagent; Anal.Biochemistry; 1985, 529-536

Ghebihiwet B., Bossones S., Erdei A and Reid K.B.M.; Reversible biotinylation of C1q with a cleavable biotinyl derivative; J.Immunol.Meth., 1979, 110, 251-260

Struchbury T., Shipton M., Norris R., Malthouse J.P.G. and Brocklehurst K.; Reporter group delivery system with both absolute and selective specificity for thiol groups and an improved fluorescent probe containing the 7-nitrobenzo-2-oxa-1.3-diazole moiety. Biochem. J. 1975, 151, 417-432

Yoshitake S., Yamada Y, Ishikawa E. and Masseyeff R.; Conjugation of glucose oxidase from Aspergillus niger and rabbit antibodies using N-Hydroxysuccinimide ester on N-(-4-carboxycyclohexyl-methyl)-maleimide. Eur.J.Biochem., 1979, 101, 395-399

Gurd F.R.N., Carboxymethylation; Meth.Enzymol. 1967, XI, 532-541

Yamamoto K., Sekine T., and Sutoh K.; Spatial relationship between SH1 and the actin binding site on myosin subfragment-1 surface; Fed.Eur.Biochem.Soc., 1984, 176, 75-78

Other information

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

Related product and documents:

▪Other Maleimide-Biotins including the PEO spacer versions PEO2 #BT3750, PEO3 #R2028A and PEO11 #BR4032)

▪SATA reagent (UP84235A) to introduce sulfhydryls

▪Desalting tools, i.e CelluSep dialysis tubings
▪PBS buffer, MES buffer
▪Preservatives: AEBS #401070 and other protease inhibitors, SodiumAzide #08112A

▪Other reactive biotins:
Succinimidyl ester NH_2-reactive biotins: ex UP52117) and the PEO-spacer versions NHS-PEOx-Biotins ex.#R2027A
Carboxylated biotins allow conjugation with amino groups. See #10685.
Aminated biotins allow conjugation with carboxyl groups. See #84961.
Hydrazide biotins, to conjugate sugars. See UP78631A.
Chromalink biotin, with UV-traceable spacer #BT3601

▪Other biotins: **2-Iminobiotin**. See #39375A.

▪See BioSciences Innovations catalogue and e-search tool.

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