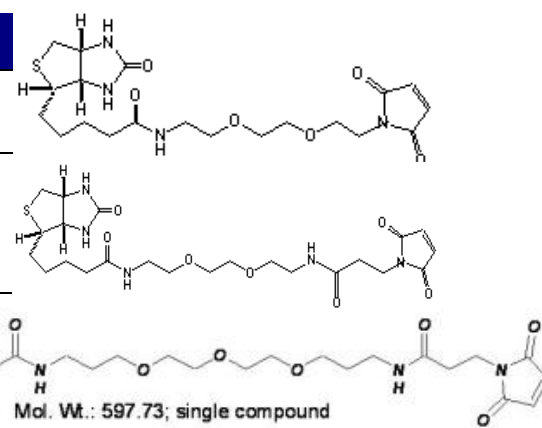
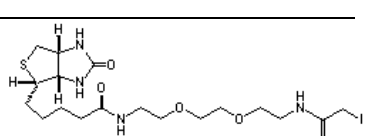


Thiols reactive Maleimido-PEO-Biotins

Products Description

PEO-structure spacer increases water solubility !

Catalog #:	Name & description	
BT3751, 50mg	Maleimido-PEO₂'-Biotin M.W.= 454.55 (K) Eq. Maleimido-PEO3-Biotin	
UP87284A, 50mg 87284B, 100mg 87284C, 500mg	Maleimido-PEO₂-Biotin M.W.= 525.62; CAS: 305372-39-8; (K) PEO tether increases water solubility! 29.1 Å long spacer	
UPR20289, 10mg UPR2028A, 25mg UPR2028B, 50mg	Maleimido-PEO₃-Biotin M.W.= 597.74; CAS: 1431618-70-0 (K) 24.9 Å long spacer	
AWJMN1	Maleimido-PEO₄-Biotin	
AWJMX1	Maleimido-PEO₆-Biotin MW :701.83 ; CAS:1808990-66-0 ; (K)	
BR4032, 50mg	Maleimido-PEO₁₁-Biotin M.W.= 922.11 (K) 50.5 Å long spacer	
AWJMJ1 (M)	Maleimido-PEO₁₂-Biotin Soluble in DMSO, DMF, water;	
AWJMQ1 (M)	Maleimido-PEO₂₃-Biotin Soluble in DMSO, DMF, water;	
AWJMD1 (M)	Maleimido-PEO₄₅-Biotin Soluble in DMSO, DMF, water;	
AWJNM1 (M)	Maleimido-PEO₇₅-Biotin Soluble in DMSO, DMF, water;	
88365A	(+)-Biotin-PEO₃-iodoacetamide MW= 542.44; mp 126-127 °C 24.7 Å long spacer	 Mol. Wt.: 597.73; single compound

See higher PEO spacer lengths as Maleimido-PEG_x-Biotin () #AWJNDA (polydisperse)

Storage: +4°C, protected from light and moisture (desiccate). Possible at -20°C for long term. (K)

Introduction

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 complexe 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, ...

FT-R2028A

- **Purification** of biotinylated molecules, or molecules having bound to a biotinylated ligand: a biotinylated molecule, or its complex, can be affinity purified from a complex mixture (detergent cell extract) with an immobilized avidin support (#UP34090A and related products) (Ghebrehiwet 1979).

Compounds like biotin-HPDP and biotin-BMCC (see related products) impart detrimental properties when labeled due to the presence of both a highly hydrophobic spacer and reactive functionality. Interchim provides biotinylation agents containing **PEO spacer** that confers hydrophilicity not only to the reagent but also to the conjugate. This gives several advantages:

- higher coupling ratio can be achieved
- reduced non-specific binding, improved bio-interactions (higher signal)
- increased biostability of the conjugate (i.e. minimizes precipitation)
- no immunogenicity

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 cysteine), and it is well stable. The competitive hydrolysis forming maleamic acid becomes noticeable when pH goes up to 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.
- **Solubility** in water is usually good up to 20mM (UP48198: 20mg/ml in acetic acid). For higher solubility, see Maleimide-PEO-Biotins: longer spacer versions PEO2 #[87284A](#), PEO3' #[BT3750](#) and PEO11 #[BR4032](#).
- **PEO Spacers** are ideal for rapid and tight avidin/streptavidin binding.
 - By its PEO nature, hydrophilic spacer arm 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) to 50.5 Angstrom (Biotin-amide carbonyl to terminal maleimide).
 - Additionally, PEO spacer are non-immunogenic.
- The **biotin** is a vitamin widely used in biotechnology for its propriety to bind with extremely high affinity to avidin ($K_a=10^{-15} \text{ M}^{-1}$) and streptavidin ($K_a=10^{-14} \text{ M}^{-1}$). 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 attachment to amines, that generally don't affect the biological activity, attachment 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 onto 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).

 - higher coupling ratio can be achieved
 - minimizes aggregation of conjugates, or conjugates/ligands complexes, hence increased biostability of the conjugate
 - no immunogenicity
 - reduced non-specific binding

Use

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

Protocol 1: 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 be 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.

Other information

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

Related products and documents:

- other SH-reactive biotins: MAL-Biotins [FT-48198A](#) (activated by Maleimide, Pyridylthio, Iodoacetamide)
- other PEO spacer Biotins: Biotin-PEO_x-PFP ester #FV0111 Biotin-PEO₄-NHSuccinimide [#R2027A](#)
Biotin-PEO₄-Hydrazide [#BJ008A](#) Biotin-PEO₄-SS-Biotin [#CC4431](#)
- Other reactive biotins:
Succinimidyl ester NH₂-reactive biotins: See [#52117A](#)
Carboxylated biotins allow conjugation with amino groups. See [#10685A](#).
Aminated biotins allow conjugation with carboxyl groups. See [#84961A](#).
Hydrazide biotins, to conjugate sugars. See [#78631A](#).
Chromalink biotin, with UV-traceable spacer [#BT3600](#).
- SATA reagent #UP84235A to introduce sulfhydryls onto amines
- Desalting tools, i.e [CelluSep dialysis tubings](#)
- [PBS buffer](#), [MES buffer](#)
- Preservatives: AEBS [#401070](#) and other protease inhibitors, SodiumAzide [#08112A](#)
- See [BioSciences Innovations catalogue](#) and [e-search tool](#).

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

213 av.J.F.Kennedy, 03103 Montluçon, fax : +33(0)4 70 03 82 60, hotline: +33(0)4 70 03 76 06

rev. H010E