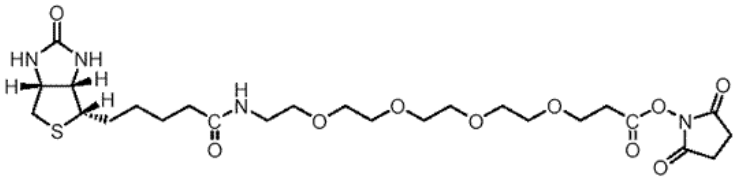
FT-R2027A

NHS-PEO$_x$-Biotin

Products Description

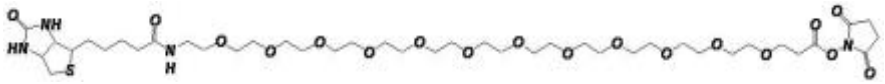
Name: **NHS-PEO$_4$-Biotin**
Reference : UPR20278, 10x2 mg
UPR2027A, 25 mg
UPR2027B, 100 mg
UPR2027C, 1g



Physical data:

MW: 588.67 19.6A spacer
Soluble 10 mg/ml in aqueous solutions, and organic solvents

ame: **NHS-PEO$_{12}$-Biotin**
Reference : BZ0971, 25mg
BZ0973, 500mg



Physical data:

MW: 941.09 spacer: 47.6A length
Soluble in aqueous solutions, and organic solvents

More:

NHS-PEG2-Biotin #B4Y9L-	
NHS-PEG3-Biotin #AWJMY-8X256-	CAS:1253286-56-4; MW: 544,6
NHS-PEG4-Biotin #B4Y8Z-AWJN1-MRU33	CAS:459426-22-3; MW:588.7
NHS-PEG6-Biotin #AYNPG-	CAS:2055045-04-8; MW: 676.7
NHS-PEG8-Biotin #8X397-	CAS:2143968-93-8; MW:764.9
NHS-PEG12-Biotin #AWJMK-	
NHS-PEG23-Biotin #AWJMR-	
NHS-PEG45-Biotin #AWJNE-	
NHS-PEG75-Biotin #Inquire	

PEO$_n$ spacer (PolyEthylOxy motif) indicates a synthetic single compound. It is also known as PEG$_n$, and dPEG, while PEG and PEG$_{xxx}$ usually refer to polydisperse purified compounds.

Storage: Store at -20°C (possible +4°C for short term) - protect from light and moisture

Introduction

The NHS-PEOx-Biotin reagents enable simple and efficient **biotin labeling of biomolecules**, antibodies, proteins and any other primary amine-containing macromolecule. Specific **labeling of cell surface** proteins is another common application for this uniquely water-soluble and membrane impermeable reagent. They are available with 2 spacer lengths, 19.6 and 47.6 Angstroms.

NHS-PEOx-Biotin replace advantageously sulfo-NHS-lc-Biotins [a], thanks the hydrophilic polyethylene oxide (PEO) spacer arm that imparts water solubility with several benefits:

- the reagent can be **dissolved directly in aquous solutions**
- the **hydrophilicity is transferred** to the biotinylated molecule
- you can achieve **higher coupled ratios**
- you **reduce eventual aggregation** of labeled proteins, when stored in solution and in binding conditions
- you **reduce non-specific binding** on surfaces
- **no immunogenicity** is conferred to the conjugate

[a]SulfoNHS-X-Biotins hydrophilicity is conferred by sulfoNHS, that is useful for solubilization in aqueous buffers, but not after coupling.

For any question, contact you local distributor
Uptima, powered by interchim
213 Avenue J.F. Kennedy - BP 1140
03103 Montluçon Cedex - France
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60
uptima@interchim.com

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Directions for use

The following protocols are example applications, that should be optimized or adapted to specific applications.

Solubilization and general information

NHS-PEOx-Biotin is moisture-sensitive. Store at 4-8°C, preferably in a desiccator. To avoid moisture condensation onto the product, vial must be equilibrated to room temperature before opening.
NHS-PEOx-Biotin can be dissolved directly in water, or pre-dissolved in organic solvent (DMSO, DMF).
Avoid buffers containing primary amines (e.g., Tris or glycine), as these will compete with the NHS-ester reaction.
Use reconstituted NHS-PEOx-Biotin immediately. The NHS-ester moiety readily hydrolyzes and becomes non-reactive, therefore, solutions cannot be prepared for storage. Discard any unused reconstituted reagent.

Example Procedure for IgG Biotinylation using NHS-PEO$_4$-Biotin

This protocol typically results in ~3-5 biotin molecules per molecule of IgG. The molar ratio of NHS-PEO$_4$-Biotin to protein may be adjusted as desired.

A. Additional Materials Required

Reaction Buffer: Phosphate Buffered Saline (e.g., Phosphate Buffered Saline Packs containing 0.1 M phosphate, 0.15 M NaCl, pH 7.2, Product #307157), or other non-amine containing buffer at pH 7.0-8.5
Method for removal of non-reacted biotin reagent (Buffer Exchange): MicroDispoDialyzer Dialysis Units for 10-100 µl sample volumes (Product # 905320); Float-A-Lyzer® Dialysis Cassette Kit for 3.0 ml sample volumes (Product # 883480); or Desalting Columns G25, 5 x 10 ml (Product # 848742)
Optional: HABA (Product #UP05361D) for quantitation of biotin (incorporation ratio).

B. Calculations

The amount of biotin reagent to use for each reaction depends on the amount of the protein to be labeled and the protein concentration. By using the appropriate molar ratio of biotin to the protein, the extent of labeling can be controlled. For dilute protein solutions (e.g., 2 mg/ml) a greater molar fold excess of biotin is used compared to a concentrated protein solution (e.g., 10 mg/ml). For example, for best results use 20-fold molar excess of biotin for a 2 mg/ml IgG solution or 12-fold molar excess of biotin for a 10 mg/ml IgG solution.

1. Calculate millimoles of NHS-PEO$_4$-Biotin to add to the reaction for a 20-fold molar excess:

$$\text{ml protein} \times \frac{\text{mg protein}}{\text{ml protein}} \times \frac{\text{mmol protein}}{\text{mg protein}} \times \frac{\text{20 mmol Biotin}}{\text{mmol protein}} = \text{mmol Biotin}$$

2. Calculate microliters of 10 mg/ml NHS-PEO$_4$-Biotin (prepared in Step C.2) to add to the reaction:

$$\text{mmol Biotin} \times \frac{\text{589 mg}}{\text{mmol Biotin}} \times \frac{\text{200 µl}}{\text{2.0 mg}} = \text{µl Biotin}$$

where 20 = recommended molar fold excess of biotin for 2 mg/ml IgG sample
589 = molecular weight of NHS-PEO$_4$-Biotin
200 = microliters of water in which 2.0 mg of NHS-PEO$_4$-Biotin is dissolved

EXAMPLE: for 1 ml of a 2 mg/ml IgG (150,000 MW) solution, 15.6 µl of 10 mg/ml NHS-PEO$_4$-Biotin will be added.
Calculations:

$$\text{1 ml IgG} \times \frac{\text{2 mg IgG}}{\text{1 ml IgG}} \times \frac{\text{1 mmol IgG}}{\text{150,000 mg IgG}} \times \frac{\text{20 mmol Biotin}}{\text{1 mmol IgG}} = \text{0.000266 mmol Biotin}$$

$$\text{0.000266 mmol Biotin} \times \frac{\text{589 mg}}{\text{mmol Biotin}} \times \frac{\text{200 µl}}{\text{2.0 mg}} = \text{15.6 µl NHS-PEO}_4\text{-Biotin}$$

C. Biotinylation reaction

For reaction volumes from 10 to 100 µl, the buffer exchange and biotinylation may be conveniently performed in a single MicroDispoDialyzer Dialysis Unit. For reaction volumes from 0.1 to 12 ml, Float-A-Lyzer® Dialysis tubes may be used.

1. Dissolve or buffer exchange the IgG into Reaction Buffer.
2. Immediately before use, add 200 µl of ultrapure water to 2 mg of NHS-PEO$_4$-Biotin.
3. Add the appropriate volume of the NHS-PEO$_4$-Biotin solution (see Calculations section) to the IgG solution.

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03103 Montluçon Cedex - France
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60
uptima@interchim.com

4. Incubate reaction on ice for two hours or at room temperature for 30 minutes.
5. Remove the non-reacted NHS-PEO$_4$-Biotin by dialysis or gel filtration. See instructions provided with preferred buffer exchange product.
6. Store the biotinylated protein using the same condition that is optimal for the non-biotinylated protein.

D. Determination of Biotin Incorporation (optional)

Biotin incorporation can be estimated using the HABA [2-(4'-hydroxyazobenzene)-benzoic acid] method (Product #UP05361D). This method is based on the ability of the HABA dye to bind avidin forming a complex with maximal absorption at 500 nm. Biotin is then added to the solution and because of its higher affinity for avidin, biotin displaces the HABA and the absorption at 500 nm decreases proportionately. The absorbance of the HABA-avidin solution is measured before and after adding the biotin-containing sample. The change in absorbance relates to the amount of biotin in the sample.

Example Procedure for Biotinylation of Cell Surface Proteins

1. Wash cells three times with ice-cold PBS (pH 8.0) to remove any contaminating proteins.
2. Suspend cells at a concentration of 25 x 10^6 cells/ml in PBS (pH 8.0).
   Note: Other cell concentrations may be used. The concentration of biotinylation reagent may be scaled up or down based on cell concentration, size or type.
3. Immediately before use, add 200 µl of ultrapure water to 2 mg of NHS-PEO$_4$-Biotin.
4. Add ~50 µl of the NHS-PEO$_4$-Biotin solution per milliliter of reaction volume.
5. Incubate reaction at room temperature for 30 minutes.
6. Wash cells three times with ice-cold PBS (pH 8.0) to remove non-reacted biotinylation reagent. Alternatively, 25-50 mM Tris (pH 8.0) may be used for the initial wash to quench any non-reacted biotinylation reagent.

Troubleshooting

Problem	Possible Cause	Solution
Lack of biotinylation	No amines available on molecule of interest	Use a biotinylation reagent that targets a different functional group or convert sulfhydryl to amine using Aminoethyl-8 (Product #24653P)
	Buffer contains primary amines	Use a non-amine containing buffer
		Extensively dialyze or desalt sample into a buffer free of primary amines
	Reagent is non-reactive caused by hydrolysis of the NHS ester	Use reagent immediately upon reconstitution
	Moisture condensation on the product vial has caused hydrolysis of the NHS ester	Purchase new reagent and always allow it to equilibrate to room temperature before opening
Biotinylated protein does not function in downstream application	Excessive biotinylation	Reduce molar excess of biotinylation reagent, or reduce time or temperature for biotinylation
		Choose biotinylation reagent that targets different groups

References

Altin, J.G., *et al.* (1995). A one-step procedure for biotinylation and chemical cross-linking of lymphocyte surface and intracellular membrane-associated molecules. *Anal. Biochem.***224:**382-9.
Gretch, D.R., Suter, M. and Stinski, M.F. (1987). The use of biotinylated monoclonal antibodies and streptavidin affinity chromatography to isolate herpes virus hydrophobic proteins or glycoproteins. *Anal. Biochem*. **163:**270-7.
Manning, J., *et al.* (1977). A method for gene enrichment based on the avidin-biotin interaction. Application to the Drosophila ribosomal RNA genes. *Biochemistry* **16:**1364-70.
Updyke, T.V. and Nicolson, G.L. (1984). Immunoaffinity isolation of membrane antigens with biotinylated monoclonal antibodies and immobilized streptavidin matrices. *J. Immunol. Meth.* **73:**83-95.

Related products

See BioSciences Innovations catalogue and e-search tool.
*Other amine-reactive biotins:
Sulfo-NHS-x-Biotin (FT-52117A)
Biotin-PEGx-Succinimide Esters (PEGx; x=200Da to 30K)(FT-B36HM1) Biotin-PEOn-PFP ester #FV011
*Other biotins : Biotin-PEO4-Maleimide #R2028A | Biotin-PEO4-Hydrazide #BJ008A | Biotin-PEO4-SS-Biotin #CC4431 | Chromalink-Biotin (PEO spacer, color tag) #CE9601) Hydrazine-PEO-Biotins

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Uptima, powered by interchim
213 Avenue J.F. Kennedy - BP 1140
03103 Montluçon Cedex - France
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60
uptima@interchim.com