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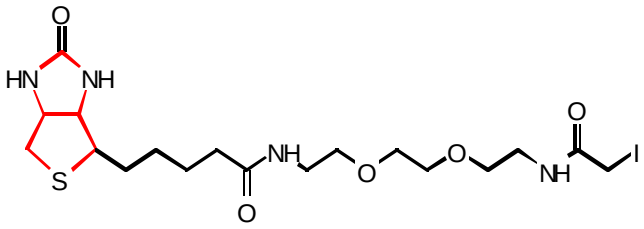
ProductInformation

Biotin Polyethyleneoxide Iodoacetamide
Biotin PEO Iodoacetamide

Product Number **B 2059**
Storage Temperature 2 - 8 °C

Product Description

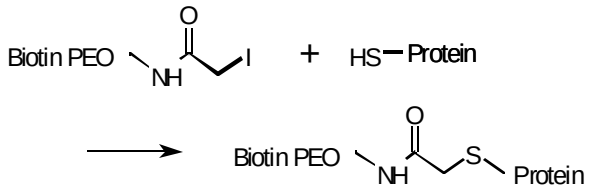


Molecular Formula: $C_{18}H_{31}IN_4O_5S$
Molecular Weight: 542.43
Purity: minimum 90% (HPLC)
Source: Synthetic

Biotin polyethyleneoxide (PEO) iodoacetamide is a sulfhydryl (thiol) specific biotinylation reagent. The iodoacetamide group reacts specifically with reduced thiols at pH 7.5 - 8.5. This allows for tagging of cysteine residues in proteins as well as conjugation to sulfhydryls introduced synthetically via amine reactive reagents such as 2-iminothiolane (Prod. No. I 6256) or S-acetylthioglycolic acid N-Hydroxysuccinimide ester (SATA, Product No. A 9043). Peptides and small molecules containing thiol groups may also be labeled using this reagent.

Due to the high affinity of biotin for the proteins avidin and streptavidin (K_a approx. 10^{15} M^{-1}), biomolecules that have been biotinylated are easily captured and/or detected using techniques such as Western blotting, ELISA, and affinity chromatography. For these applications, a wide variety of avidin and streptavidin conjugates are available including agaroses, coated multiwell plates, and enzyme conjugates. The biotin PEO iodoacetamide reagent is especially useful in many proteomics type applications such as peptide mapping, phosphopeptide analysis, and mass spectrometry.[1-3] By specifically targeting the cysteine residues of a protein for modification, a tryptic digest of a proteome can be quickly fractionated by affinity capture allowing for simplification of a complex mixture and easier identification of peptides by mass spectrometry.

The labeling of thiols with this reagent results in a stable thioether linkage:



In some cases, the target compound of interest may need to be reduced and/or denatured to create a reactive sulfhydryl group prior to biotinylation. Examples include antibodies or other proteins whose cysteine residues are involved in disulfide linkages. In other cases, the reactivity of a protein thiol may be limited due to its location in the interior of the tertiary protein structure.

Since the thiol primarily reacts as the unprotonated thiolate anion, the reactivity of a particular sulfhydryl toward the iodoacetamide group is dependent on its pK_a. A typical cysteine SH group has a pK_a of 8.5 –9.0. In a protein, this pK_a may be significantly altered due to the presence of acidic or basic residues in close proximity to the cysteine.[4]

Precautions and Disclaimer

This product is for laboratory research use only. Please consult the Material Safety Data Sheet for handling recommendations before working with this material.

Preparation Instructions

Biotin PEO iodoacetamide is soluble to 10 mg/ml in most polar solvents including, but not limited to, water, DMF, DMSO, and methanol. Brief sonication or vortexing may aid dissolution. For use in protein labeling, it is recommended to prepare a 5 - 10 mg/ml solution in water. This solution should be protected from light at all times.

Storage/Stability
The product as a solid should be stored protected from light and moisture at 2 - 8 °C. Once reconstituted in water, the product is stable for at least 4 hours at room temperature when protected from light. In the presence of light, molecular iodine can form which may react with tyrosine residues in a protein.

Procedure for sample preparation (reduction/denaturation) prior to biotinylation)
1. Dissolve protein of interest at approximately 2 mg/ml in a buffer of choice at pH 7.5 – 8.5. Recommended buffer systems include 50 mM HEPES, pH 7.5, or 50 mM Tris, pH 8.5. These buffers may also be supplemented to 1 to 5 mM with EDTA to help prevent reoxidation of reduced thiols. This buffer may also need to contain a chaotrope such as urea or guanidine HCl in order to denature the protein to ensure all disulfide bridges are accessible for reduction.
2. Add a reducing agent such as Tris(carboxyethyl)-phosphine (TCEP, Prod. No. C 4706) or Tributylphosphine (TBP, Prod. No. T 7567) to a final concentration of 5 mM. Stir for 60 minutes at room temperature.
3. Dialysis or gel filtration chromatography may be performed at this step to remove denaturant and/or reducing agent, but is not necessary. Handle the sample quickly to prevent reoxidation of thiol groups.

Procedure for protein biotinylation
1. Dissolve Biotin PEO iodoacetamide at 5 to 10 mg/ml in water (9.2 –18.4 mM). Use an amber vial or wrap container in foil to protect from light.
2. Reconstitute protein of interest in a buffer of choice pH 7.5 - 8.5 to approximately 2 mg/ml. Recommended buffer systems include 50 mM HEPES, pH 7.5, or 50 mM Tris, pH 8.5. These buffers may also be supplemented to 1 to 5 mM with EDTA to help prevent reoxidation of reduced thiols. See sample preparation procedure if reduction of disulfides and/or denaturation is required before biotinylation.
3. If sulfhydryl content of protein solution is known, add a 2 - 5 molar excess Biotin PEO iodoacetamide. If sulfhydryl content of protein solution is not known, add Biotin PEO iodoacetamide to a final concentration of 2 mM. This assumes a protein concentration of 2 mg/ml, an average protein mass of 30 kDa and 6 cysteines/protein.
4. Stir gently, protected from light for 2 - 4 hours at room temperature.
5. Excess biotinylation reagent may be removed by gel filtration chromatography or by dialysis with an appropriate molecular weight cut-off membrane.
6. In mass spectrometry (MS) applications, the protein may be digested with proteomics grade trypsin (Prod. No. T 6567) before affinity purification and analysis by MS.
7. Capture the biotinylated protein or biomolecule using streptavidin agarose (Prod. No. S 1638), monomeric avidin agarose (Prod. No. A 2036), streptavidin high capacity coated plates (Prod. No. S 6940), or streptavidin coated magnetic beads (Prod. No. S 2415). Alternatively, the protein may be detected in an ELISA or Western blot procedure using streptavidin alkaline phosphatase (Prod. No. S 2890) or streptavidin peroxidase (Prod. No. S 5512) conjugates.

Results
The protein concentration of a sample may be determined prior to biotinylation using a BCA assay (e.g. Prod. Code BCA-1).
The sulfhydryl content of a protein sample may be quantitated using Dithiobis(2-nitrobenzoic acid) (DTNB, Prod. No. D 8130).[5]
The level of protein biotinylation may be quantitated using the HABA/Avidin assay (e.g. Prod. No. H 2153).[6]

Specificity
The iodoacetamide group is generally considered to specifically react with thiols. If there are no sulfhydryl compounds present, however, the iodoacetamide group may react with histidines, methionines, and amines.[7] The reaction rate of iodoacetamide with sulfhydryls is much faster than any of the other potentially reactive groups. The specificity toward thiols may generally be controlled by limiting the amount of labeling reagent used and using the lowest pH necessary for the reaction. The iodoacetamide group will react with methionine under acidic conditions.[8]

References

1. Griffen, T.J. and Aebersold, R., Advances in Proteome Analysis by Mass Spectrometry, J. Biol. Chem., **276**, 45497-45500 (2001).
2. Goshe, M.B., et al., Phsophoprotein isotope-coded affinity tag approach for isolating and quantitating phosphopeptides in proteome-wide analyses, Anal. Chem., **73**, 2578-2586 (2001).
3. Conrads, T.P., et al., Quantitative Analysis of Bacterial and Mammalian Proteomes Using a Combination of Cysteine Affinity Tags and ^{15}N-Metabolic Labeling, Anal. Chem., **73**, 2132-2139 (2001).
4. Britto, P.J., et al., The Local Electrostatic Environment Determines Cysteine Reactivity of Tubulin, J. Biol. Chem., **277**, 29018-29027 (2002).
5. Ellman, G.L., Arch. Biochem, Biophys., **82**, 70 (1959).
6. Green, N.M., Methods Enzymol., **18A**, 418-424 (1970).
7. Gurd, F.R.N., Carboxymethylation, Methods Enzymol., **11**, 532-541 (1967).
8. Stark, G.R., et al., Relationships between the conformation of Ribonuclease and Its Reactivity toward Iodoacetate, J. Biol. Chem., **236**, 436-442 (1961).

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