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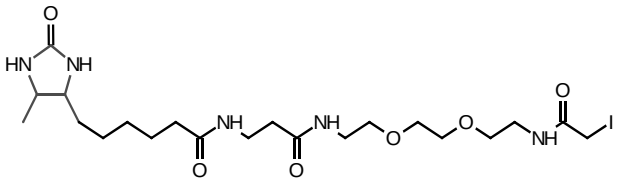
ProductInformation

Desthiobiotin Polyethyleneoxide Iodoacetamide
Desthiobiotin PEO Iodoacetamide

Product Code **D 2192**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description



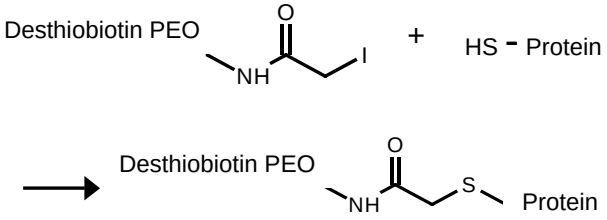
Molecular Formula: $C_{21}H_{38}IN_5O_6$
Molecular Weight: 583.5
Purity: minimum 90% (HPLC)

Desthiobiotin polyethyleneoxide (PEO) iodoacetamide is a sulfhydryl (thiol) specific labeling 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 (Product Code I 6256) or S-acetylthioglycolic acid N-Hydroxy-succinimide ester (SATA, Product Code A 9043). Peptides and small molecules containing thiol groups may also be labeled using this reagent.

Desthiobiotin has a strong affinity for the proteins avidin and streptavidin (K_d approx. 10^{-11} M).[1] Biomolecules that have been desthiobiotinylated can be quickly and quantitatively captured using affinity chromatography or detected using techniques such as Western blotting and ELISA. For these applications, a wide variety of avidin and streptavidin conjugates are available including agaroses, coated multiwell plates, and enzyme conjugates. Because the binding affinity is reduced compared to traditional biotin derivatives, the captured biomolecules can be efficiently eluted from immobilized streptavidin using competitive displacement under mild conditions.[2] These conditions generally consist of a low concentration of biotin at a neutral pH. In contrast, biotinylated compounds can only be efficiently eluted using extremely harsh conditions of low pH in the presence of denaturants or organic solvents.

Desthiobiotin PEO iodoacetamide is especially useful in many proteomics type applications such as peptide mapping, phosphopeptide analysis, and mass spectrometry.[3-6] By specifically targeting the cysteine residues 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 desthiobiotinylation. 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 to 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.[7]

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
Desthiobiotin PEO iodoacetamide is soluble to at least 10 mg/ml in most polar solvents including, but not limited to, water, DMF, DMSO, and methanol. 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 should be stored as a solid 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. If desired, the aqueous solution may be frozen at –20 °C in aliquots. The frozen solution is stable for at least one week.

General procedure for sample preparation (reduction/denaturation) prior to desthiobiotinylation
1. Dissolve the 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. The 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, Product Code C 4706) or Tributylphosphine (TBP, Product Code T 7567) to a final concentration of 5 mM. Stir for 30 to 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 always necessary. A separation step is especially important if TCEP is used in step 2 since it will react with iodoacetyl groups. TBP is the preferred reducing agent if this separation step is skipped. Handle the sample quickly to prevent reoxidation of thiol groups.

Procedure for protein desthiobiotinylation
1. Dissolve Desthiobiotin PEO iodoacetamide at 5 to 10 mg/ml in water (8.6 – 17.1 mM). Use an amber vial or container wrapped 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 - 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 desthiobiotinylation.
3. If the sulfhydryl content of protein solution is known, add a 2 - 5 molar excess of Desthiobiotin PEO iodoacetamide. If sulfhydryl content of protein solution is not known, add Desthiobiotin 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 per protein molecule.
4. Stir gently, protected from light for 2 - 4 hours at room temperature.
5. Excess desthiobiotinylation reagent may be removed by gel filtration chromatography or dialysis with an appropriate molecular weight cut-off membrane.
6. In mass spectrometry (MS) applications, the protein may be digested with proteomics grade trypsin (Product Code T 6567) before proceeding to affinity purification.
7. Capture the desthiobiotinylated protein or peptides using streptavidin agarose (Product Code S 1638), avidin agarose (Product Code A 9207), streptavidin high capacity coated plates (Product Code S 6940), or streptavidin coated magnetic beads (Product Code S 2415). Alternatively, the protein may be detected in an ELISA or Western blot procedure using streptavidin alkaline phosphatase (Product Code S 2890) or streptavidin peroxidase (Product Code S 5512) conjugates. Binding should be performed in a neutral buffer (50 mM ammonium phosphate, pH 7.4) for 15 minutes at room temperature.
8. Wash the streptavidin/avidin matrix as recommended to remove non-biotinylated molecules.
9. Elute desthiobiotinylated biomolecules using 2 – 5 mM biotin (Product Code B 4501) in 25 mM ammonium phosphate, pH 7.4. Incubate for 15–60 minutes at room temperature. The addition of 10–50% MeOH to the elution solution greatly accelerates the elution process, but is not necessary. For example, in 50% MeOH with 5 mM biotin, 100% of a bound desthiobiotinylated molecule eluted in less than 5 minutes. Without MeOH, the same elution with 5 mM biotin requires up to 1 hour incubation for quantitative recovery.

Results

The protein concentration of a sample may be determined prior to desthiobiotinylation using a BCA assay (Product Code BCA-1).

The sulfhydryl content of a protein sample may be quantitated using Dithiobis(2-nitrobenzoic acid) (DTNB, Product Code D 8130).[8]

The level of desthiobiotinylation of a protein may be quantitated using the HABA/Avidin assay (Product Code H 2153).[1]

Specificity

The iodoacetamide group is generally considered to specifically react with thiols. However, if there are no sulfhydryl compounds present, the iodoacetamide group may react with histidines, methionines, and amines.[9] The reaction rate of iodoacetamide with sulfhydryls is much faster than any of the other potentially reactive groups. The specificity towards 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.[10]

References

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