

Product Data Sheet

Product Name: Biotin-HPDP

Cat. No.: GC11037

Chemical Properties

Cas. No. 129179-83-5

Chemical Name 5-[(3aS,4S,6aR)-2-oxo-1,3,3a,4,6,6a-hexahydrothieno[3,4-d]imidazol-4-yl]-N-[6-[3-(pyridin-2-yl)disulfanyl]propanoylamino]hexyl]pentanamide

SMILES C1C2C(C(S1)CCCCC(=O)NCCCCCNC(=O)CCSSC3=CC=CC=N3)NC(=O)N2

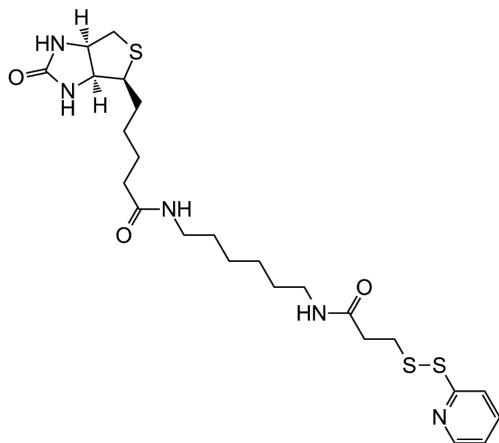
Formula $C_{24}H_{37}N_5O_3S_3$ M.Wt 539.78

Solubility 5.4mg/mL in DMSO (ultrasonic and warming and heat) Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution : ship with blue ice All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

Evaluation of assessment of sulphydration using Biotin-HPDP

This protocol only provides a guideline, and should be modified according to your specific needs

1. Stock and dilute solution preparation for staining of cells

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(1) Prepare Biotin-HPDP stock solution (50mM) by dissolving Sulfo-NHS-SS-Biotin powder into DMSO.

(2) Dilute the Biotin-HPDP stock solution with DMSO to a final 0.8mM concentration. NOTE: The working solution must be prepared fresh on the day of the experiment.

2. Cell Preparation

Seed cultured cells in a 6-well plate or a 60-mm culture dish, depending on the scale of the experiment. If the cells tend to detach easily, precoat the plate or dish with poly-l/d-lysine to enhance cell adhesion.

3. Biotin Labeling on the Cell Surface

(1) Incubate the plates on ice for 10 minutes. This step stops further trafficking or endocytosis, ensuring that only proteins currently present on the cell membrane are labeled with biotin.

(2) Wash the cells twice with ice-cold PBS.

(3) Add 0.8mM of Biotin-HPDP to the plates and incubate at 4°C for 30 minutes in the dark with gentle agitation.

(4) Wash the cells twice with PBS, then incubate them with PBS containing 1% bovine serum albumin at 4°C for 10 minutes to quench any unbound biotin. A 50mM glycine solution in PBS can also be used for quenching the reaction.

(5) Wash the cells twice with PBS before harvesting to remove any remaining quenching buffer.

(6) Collect cells into a 1.5-ml tube with lysis buffer containing 150mM NaCl, 50mM Tris-HCl (pH 7.4), 1mM EDTA, 1% Triton X-100, and protease inhibitor cocktail. Routinely use 200-300µl lysis buffer per one 6-well plate sample. To break cell membranes and homogenize the sample, briefly sonicate the cells. Transfer the supernatant to a new 1.5-ml tube.

4. Streptavidin-Biotin Incubation

(1) Add 50µl of 50% Streptavidin resins to a 1.5-ml tube. Gently resuspend the streptavidin beads by inverting or vortexing the bead suspension. Make sure the beads are fully mixed before use.

(2) Equilibrate the beads by incubating them with 1ml of lysis buffer on an end-over-end rotator at 4°C

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for 1 hour. Briefly centrifuge the tube on the tabletop centrifuge and aspirate the supernatant solution. Repeat the equilibration wash once more. After the final wash, use a gel-loading tip to carefully and completely remove any remaining lysis buffer.

(3) Add the cell lysate to the beads. Then, add an appropriate amount of lysis buffer to bring the total volume to 200µl per sample.

(4) Incubate the lysate-streptavidin mixture on an end-over-end rotator at 4°C for 4 hours to overnight.

(5) Use a tabletop centrifuge to aspirate the supernatant solution. Add 1ml lysis buffer and wash on an end-over-end rotator at 4°C for 5 minutes. Repeat the wash twice more. After the final wash, use a gel-loading tip to carefully and completely remove the remaining lysis buffer.

5. Elution, Visualization, and Quantification

(1) Elute biotinylated proteins in sample buffer containing 225mM Tris-HCl (pH 8.45), 6% glycerol, 2% sodium dodecyl sulfate, and 65mM dithiothreitol. Incubate at 37°C for 60 minutes.

(2) Resolve the eluted sample by SDS-PAGE and carry out immunoblotting.

6. Experimental Precautions

(1) Because Biotin-HPDP is prone to hydrolysis, dissolve the Biotin-HPDP in DMSO immediately before use, and ensure that the biotin solution is adequately protected from light.

(2) This protocol and all reagents are for scientific research use only.

References:

[1] Paul B D, Snyder S H. Protein sulfhydration[M]//Methods in enzymology. Academic Press, 2015, 555: 79-90.

Background

Biotin-HPDP is a sulfhydryl-reactive biotinylation reagent, which is used to label protein cysteines and the substrates that contain sulfhydryl groups^[1]. Biotin-HPDP can be used to detect S-Nitrosation in proteins and to reduce the occurrence of false positive signals^[2]. Biotin-HPDP has been widely used in the fabrication of micro cantilever sensors and in the study of the binding interaction between biotin and streptavidin^[3].

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References:

- [1] Paul B D, Snyder S H. Protein sulfhydrylation[M]//Methods in enzymology. Academic Press, 2015, 555: 79-90.
- [2] Huang B, Chen C. An ascorbate-dependent artifact that interferes with the interpretation of the biotin switch assay[J]. Free Radical Biology and Medicine, 2006, 41(4): 562-567.
- [3] Shu W, Laue E D, Seshia A A. Investigation of biotin–streptavidin binding interactions using microcantilever sensors[J]. Biosensors and bioelectronics, 2007, 22(9-10): 2003-2009.

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