

Biotin Hydrazide Protocol and Product Information Sheet

Product Category: Biotinylation Reagents

Catalog Number(s): <u>b2106-100mg</u>, <u>b2106-1g</u>, b2106-custom

Product Name: Biotin Hydrazide

Alternative Name(s): (+)-Biotin Hydrazide; Biotin-Hz; Hydrazide Biotin; Biotine Hydrazide

CAS Number: 66640-86-6 Chemical Formula: $C_{10}H_{18}N_4O_2S$ Molecular Weight: 258.34 Spacer Length: 15.7 Å

Storage: Upon receipt store at 4°C (shipped at ambient temperature).

Product Information

Biotin Hydrazide (Biotin-Hz) is most commonly used for biotinylating carbohydrate moieties after they are oxidized to the aldehyde, forming a hydrazone linkage. Biotin Hydrazide can also be used to biotinylate carboxylic acid groups, with the use of EDC-HCI (Product # c1100).

Notes: Avoid using primary amine containing buffers, such as Tris.

Biotin Hydrazide Glycoprotein Biotinylation Protocol

- Immediately before use, dissolve Sodium meta-Periodate (<u>cr8103-5gm</u>) at a concentration of 20 mM in 100mM Sodium Acetate, pH 5.5. Store in crushed ice until use.
- 2. Dissolve glycoprotein at a concentration of 1-5 mg/mL in cold 100mM Sodium Acetate, pH 5.5.
- 3. Gently mix the glycoprotein solution with the periodate solution (1:1) (0.5mL to 1mL of each is a typical volume).
- 4. Allow reaction to proceed at 0-4°C for 30 minutes.
- 5. Desalt the oxidized glycoprotein through gel filtration (spin format), with a resin such as Sephadex® G-25 to remove excess periodate. Column should first be equilibrated with 50-100 mM Sodium Phosphate buffer, pH 7.0-7.5 to exchange oxidized glycoproteins to the appropriate buffer for conjugation.
- 6. Dissolve Biotin Hydrazide in DMSO (cr8105-25ml) at 25-50mM.
- Once the glycoprotein has been desalted, add sufficient biotinylating reagent to give a 5-10mM Biotin Hydrazide concentration.
- 8. Allow biotinylation reaction to proceed for 2 hours at room temperature.
- 9. Remove unreacted material through dialysis or gel filtration with a resin, such as Sephadex® G-25.

References:

Bayer, E.A., Ben-Hur, H., Wilchek, M. *Anal. Biochem.* 1988. 170, 2, 271-281.

O'Shannessy, D.J., Quarles, R.H. *J. Immunol. Meth.* 1987, 99, 153-161.

Hermanson, G.T. 1996. *Bioconjugate Techniques*. Academic Press, San Diego, CA, USA.

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