(+)-Biotinamidohexanoic acid hydrazide

*BACH; (+)-Biotin-X-hydrazide, Biotinamidocaproyl hydrazide, Hydrazide-LC-Biotin*

*Product No. A7821*

**Description**

**Formula:** \( C_{16}H_{29}N_{5}O_{3}S \)

**Molecular weight:** 371.50 g/mol

**CAS-No.:** [109276-34-8]

**Length of Spacer Arm:** 22.4 Å

**Melting point:** 195-199°C

**Storage:** -20°C; under argon

Store with dessicant. To avoid moisture condensation, equilibrate vial to room temperature before opening.

**Stability:** two years

**Solubility:** soluble in DMF (20 mg/ml) and DMSO

Slightly soluble in water.

**GENERAL PRODUCT INFORMATION**

- Reactivity: Hydrazide-LC-Biotin reacts with CHO (aldehyde) groups created by periodate oxidation of carbohydrates forming a hydrazone linkage.

- The coupling takes place in aqueous buffers at pH 4 - 7.4.

  **Note:** *Avoid Tris buffer or other amine containing buffers as they react with aldehydes!*

- Biotin hydrazide is soluble in aqueous buffer up to 5 mM. It may be dissolved in DMSO (50 mM) before being added to aqueous buffers for coupling or added directly as a solid to the oxidized glycoprotein solution.

- After coupling, the hydrazone linkage can be stabilized by the reduction with sodium cyanoborohydride.

- Labels Immunoglobulins in the Fc region with full retention of binding activity.

- Long spacer arm helps prevent steric hindrances.

**Applications**

- Selective binding of cell surface proteins [3]

- Labelling of endotoxin for receptor binding studies [4]

- Biotinylation of hyaluronan hydrophobic proteins and peptides [5]

- See also References arranged in chronological order.
PROTOCOL for the Biotinylation of an Antibody or a Glycoprotein

1. Add 1 ml of cold sodium periodate solution (10 mM) in PBS buffer (0.1 M pH 7.4) to 1 ml of cold IgG or glycoprotein solution (2 mg/ml) in PBS buffer (0.1 M pH 7.4); mix well and allow the oxidation to proceed for 30 min at 0 - 4°C in the dark.

2. Add glycerol to a final concentration of 15 mM, mix and incubate for 5 min at at 0 - 4°C to stop the oxidation.

3. Dialyze samples overnight against PBS buffer (0.1 M, pH 7.4) or use a desalting column equilibrated with PBS buffer (0.1 M, pH 7.4).

4. Add Hydrazide-LC-Biotin to a final concentration of 5 mM and mix.

5. React for 2 h at room temperature.

6. To reduce the hydrazone bonds to more stable linkages, cool the solution to 0°C and add an equal volume of 30 mM sodium cyanoborohydride in PBS. Incubate for 40 min.

   **Note:** If the presence of a reducing agent is detrimental to protein activity, skip this step.

7. Purify the biotinylated protein from excess reagent and by-products by gel filtration (i.e. Sephadex G-25) or dialysis against PBS.

8. Determine the degree of biotinylation with the HABA (4'-Hydroxyazobenzene-2-carboxylic acid) dye assay, described in the **APPENDIX** (Green, N.M. A spectrophotometric assay for avidin and biotin based on binding of dyes by avidin, *Biochem. J.* 94, 23c-24c (1965)).

9. Biotinylated samples may be stored using the same conditions as for the nonbiotinylated sample. A typical storage condition is 4°C for several weeks.

PROTOCOL for Coupling of Hydrazide-LC-Biotin to Carboxyl Groups

1. Dissolve protein in 0.1 M MES buffer (pH 4.7 - 5.5) at 5 - 10 mg/ml.

2. Prepare a solution of EDC (1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride) in 0.1 M MES buffer, pH 4.7 - 5.5.

3. Prepare a 50 mM Hydrazide-LC-Biotin solution in DMSO (18.6 mg /ml).

4. Add 25 µl of the Hydrazide-LC-Biotin solution to 1 ml of the protein solution and mix.

5. Add 12.5 µl of the EDC solution and mix.

6. Incubate 2 hours to overnight at room temperature.

7. Remove any precipitate that forms by centrifugation.

8. Dialyze samples overnight against PBS buffer (0.1 M, pH 7.4) or use a desalting column equilibrated with PBS buffer (0.1 M, pH 7.4).

9. Determine the degree of biotinylation with the HABA (4'-Hydroxyazobenzene-2-carboxylic acid) dye assay, described in the **APPENDIX** (Green, N.M. A spectrophotometric assay for avidin and biotin based on binding of dyes by avidin, *Biochem. J.* 94, 23c-24c (1965)).

10. Biotinylated samples may be stored using the same conditions as for the non-biotinylated sample. A typical storage condition is 4°C for several weeks.
Determination of Biotin incorporation with the HABA dye assay

1. Dissolve avidin in 0.05 M sodium phosphate, 0.15 M NaCl, pH 6.0 at a concentration of 0.5 mg/ml. 3 ml of avidin solution are needed to generate a standard curve using known concentrations of biotin. An additional 3 ml of solution are required for each sample determination.

2. Prepare a solution if 10 mM (2.42 mg/ml) of HABA (4'-Hydroxyazobenzene-2-carboxylic acid) in 10 mM NaOH. About 100 µl of HABA solution is required for each 3 ml of avidin solution.

3. Dissolve the biotinylated protein to be measured in 0.05 M sodium phosphate, 0.15 M NaCl, pH 6.0. Adjust the concentration to 10 - 20 mg/ml. Each determination requires 100 ml of sample.

4. Prepare a 0.50 mM (0.122 mg/ml) solution of D-Biotin in 0.05 M sodium phosphate, 0.15 M NaCl, pH 6.0.

5. Add a small amount of sample to the sample cuvette. Mix well and measure the absorbance at 500 nm.

6. Generate a standard curve of absorbance vs. biotin concentration. Empty the sample cell and refill it with 3 ml of the avidin solution plus 75 µl of the HABA solution to the sample cuvette. Mix well and measure the absorbance at 500 nm.

7. Next, add 3 ml of the Biotin solution. Mix well after each addition and read the absorbance at 500 nm. With each addition of biotin, the absorbance of the avidin-HABA complex decreases. To generate the standard curve, the absorbance readings are plotted against the amount of biotin added.

8. To measure the level of Biotin incorporation, add 3 ml of the avidin solution plus 75 µl of the HABA solution to the sample cuvette. Mix well and record the absorbance at 500 nm. Add a small amount of biotinylated sample. Record again the absorbance at 500 nm. Add more sample, if necessary to achieve a significant decrease in absorbance and measure again.

9. Determine the amount of biotin present in the sample by using the standard curve (steps 6 - 7).

10. The number of biotinylated sites on each molecule is obtained by dividing the number of moles of biotin by the moles of labelled molecule.

11. Sterically hindered biotinylation sites in proteins may not be able to interact with avidin. Such sites can be detected using the HABA dye assay by first subjecting the biotinylated protein to proteolytic digestion as follows: prepare a 1 % (w/v) solution of pronase in water; heat 100 µl of the sample to 56°C for 10 min, then add 10 µl of the pronase solution and incubate at room temperature overnight.

REFERENCES


