N-(5-Aminopentyl)biotinamide trifluoroacetate salt

Biotin-cadaverine

A7788

Description

The spacer arm of this biotinylation reagent is 18.9 Å providing an optimal length for efficient coupling and sensitive detection. Molecular weight is 442.50 g/mol.

The amino (-NH₂) group of this reagent can be reacted with carboxyl (-COOH) groups such as carboxy termini, aspartate and glutamate residues. The coupling requires the activation of the carboxyl group by a water soluble carbodiimide, such as EDAC. EDAC-activated carboxyl groups bind the amino group from the biotinylating re-agent, forming a stable amide bond. The conjugation yield is greatly improved if the EDAC activated carboxyl group are first converted in situ to sulfo-N-hydroxysuccinide esters by the addition of the sodium salt of 3-sulfo N-hydroxysuc-cinimide.

Polymerization of a peptide or of a protein may result if the molecule has both car-boxyl and amines on its surface. Decreasing the amount of EDAC in the reaction and/or increasing the amount of biotinylating reagents can help minimize the extent of polymerization.

EDAC reactions are generally performed in MES buffer at pH 4.5-5.5. Avoid buffers containing primary amines (Tris, glycine) or carboxyls (acetate, citrate) because they will quench the reaction. Phosphate buffers can be used, but reduce conjugation efficiency.

Solubility: Relatively water soluble; soluble in DMF and DMSO.

Biotin cadaverine is slightly water soluble. It must be dissolved in basic buffer before use in coupling to carboxylic acid groups. Alternatively, it can first be dissolved in water and subsequently the solvent is removed to obtain the free amine.

Applications: Used for colorimetric assays for factor XII carboxyls and cellular transglutaminase

See also References arranged in chronological order.

PROTOCOLS

Protocol 1: for the Biotinylation of BSA

1. Prepare a 50 mM (16.5 mg/mL) solution of 5-(Biotinamido)pentylamine in 0.1 M MES buffer, pH 4.7-5.5
2. Dissolve BSA in 0.1 M MES buffer, pH 4.7-5.5 at 5.0 mg/mL.
3. Mix equal volumes (i.e. 0.5 mL) of BSA and biotinylating reagent.
4. Add 50 μL of a 20mg/mL EDAC solution in 0.1 M, pH 4.7-5.5, MES buffer, prepared im-mediately before use.
5. Incubate for 2 hours at room temperature with stirring.
6. Remove any precipitate that formed during the reaction by centrifugation.
7. Remove non-reacted biotinylation reagent and EDAC bi-products by deasalting or dialy-sis
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 non-biotinylated sample. A typical storage condition is °4 C for several weeks.
Protocol 2: for Assaying Factor XIII in Plasma in Microwell Plates

Materials:

- Citrated Human Plasma. Collect blood in vacutainer tubes containing 38g/L sodium citrate 8one part of sodium citrate to nine parts of blood). Centrifuge at 2,500g for 10 min at °4 C. Analyze within 8 hours of collection.

- Human Thrombin Solution. Reconstitute human thrombin to 250 NIH units/mL with 25% glycerol/75% water (v/v), pH 7.4 (1,000 NIH units/mg protein). Stable at -20 °C for five months.

- N,N-Dimethylcasein solution (bovine). Prepare a 10 g/L solution in Tris-buffered saline (TBS, 40 mM Tris, pH 8.3, 150 mM NaCl) containing 50 mM DTT. Stable at -20 °C for five months.

- Biotin Cadaverine Solution. Prepare a 5 mM Biotin-cadaverine solution in TBS.

- CaCl2 solution. Prepare 5° 0 mM CaCl2 solution in TBS.

- EDTA Solution. Prepare a 200 mM EDTA solution in TBS.

- Wash Buffer. 10 mM sodium phosphate, 2.7 mM potassium chloride, 120 mM sodium chloride, pHG 7.4, 2.0 mM 2-mercaptoethanol, 0.5 g/L Tween-20, 0.5 g/L sodium azide.

- Streptavidin-β-galactosidase Solution. Immediately before use prepare a 1 μg/mL solution in Wash Buffer.

- PNPG Solution. Immediately before use prepare a 1 g/L p-nitrophenyl-b-galactopyranoside in 50 mM sodium phosphate, pH 7.2, 1.5 mM MgCl2.

Protocol 3: for Assaying Factor XIII in Plasma in Microwell Plates

Methods:

A. Biotin Incorporation into Substrate
1. Add bentonite to tubes containing citrated plasma to a final concentration of 40mg/ml.
2. Incubate for 10 minutes and centrifuge at 9,000 g for 1 min.
3. Transfer 50 μL of supernatant from each sample to microplate wells and add 10 μL of thrombin solution. Incubate for 20 min.
4. Add 20 μL of CaCl2 solution, 20 μL of N,N-Dimethylcasein solution and 100mL of 5-(Biotinamido)pentyamine solution to each well containing the sample or control. Substitute 20 μL of EDTA solution for the CaCl2 solution for controls. Mix well.
5. Incubate for 40 min with mixing
6. Quench the reaction by adding 20 μL of EDTA Solution.

B. Biotin Incorporation into Substrate
1. Add 200 μL of solution from step A6 into wells of a new microplate. Incubate for 60 min with shaking.
2. Decant the liquid and wash the wells 3 x 10 min with Wash Buffer.
3. Add 150 μL of Streptavidin-β-galactosidase Solution to each well and incubate for 30 min with shaking.
4. Decant the liquid and wash the wells 3 x 10 min with wash buffer.
5. Add 200 μL of the PNPG Solution and incubate for 20 min.
6. Measure the absorbance at 405 nm. Blanks should give an absorbance of less than 0.01
Appendix

Protocol 4: L for the 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 buffer at a concentration of 0.5 mg/mL. 3 mL of avidin solution are needed to create a standard curve using known concentrations of biotin. An additional 3 mL of solution are required for each sample determination.

2. Prepare a 10 mM (2.42 mg/mL) of HABA (4’-hydroxyazobenzene-2-carboxylic acid) in 10 mM Na-OH. 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 buffer. Adjust the concentration to 10-20 mg/mL. Each determination requires 100 μL 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. Set the spectrophotometer at 500 nm. Fill both the sample and the reference cuvette with the 0.05 M sodium phosphate, 0.15 M NaCl, pH 6.0 buffer. Zero the instrument.

6. Construct 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 aliquot 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 construct 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 at 56 °C for 10 min, then add 10 μL of the pronase solution and incubate at room temperature overnight.
References