Protocol for Labeling Glycoproteins with Hydrazide-Biotin Reagents

Note: For best results, optimize the molar ratio of reagent and glycoprotein by empirical testing.

A. Materials Required

1. Hydrazide-Biotin Solution: 50 mM hydrazide-biotin reagent in dimethylsulfoxide (DMSO).
2. Oxidation Buffer: 0.1M sodium acetate buffer, pH 5.5.
3. Sodium *meta*-periodate solution: 20 mM sodium *meta*-periodate in Oxidation Buffer. Prepare solution immediately before use in amber vial or other light-protecting vessel.
4. Coupling Buffer: 0.1 M sodium phosphate, 0.15 M NaCl, pH 7.2 (PBS) or other neutral or slightly alkaline, non-amine buffer.
5. Glycoprotein Solution: 2 mg/mL of glycoprotein in Oxidation Buffer.
6. Dialysis cassette or desalting column.

B. Procedure

1. Add 1 mL of cold sodium *meta*-periodate solution to 1mL of cold glycoprotein solution; mix well and then protect reaction vessel from light and incubate mixture for 30 minutes on ice or at 4°C.
   Note: To oxidize only sialic acid groups, add 50 µL of sodium *meta*-periodate instead of 1mL (results in 1 mM periodate final concentration rather than 10 mM).
2. Remove excess periodate and exchange the sample buffer by dialysis against coupling buffer or gel filtration through a desalting column that has been equilibrated with coupling buffer.
3. Add 1 part prepared 50 mM Hydrazide-Biotin Solution to 9 parts oxidized and buffer-exchanged sample (results in 5 mM Hydrazide Biotin); mix for 2 hours at room temperature.
   Note: Optimal hydrazide-biotin concentration and reaction conditions depend on target protein and downstream application and must be determined empirically.
4. Separate the biotinylated molecule from non-reacted material by dialysis or gel filtration (desalting column). Biotinylated samples may be stored using the same conditions as for the non-biotinylated sample.

Protocol for Labeling Carboxyl Groups with Hydrazide-Biotin Reagents

Note: For best results, optimize the molar ratio of reagents and carboxylate molecule by empirical testing.

A. Materials Required

1. Hydrazide-Biotin Solution: 50 mM hydrazide-biotin reagent in dimethylsulfoxide (DMSO).
2. MES Buffer: 0.1 M MES [(2-*N*-morpholino) ethanesulfonic acid], pH 4.7-5.5.
3. EDC (1-Ethyl-3-[3-Dimethylaminopropyl]carbodiimide Hydrochloride) solution: 100 mg/mL EDC in MES Buffer (results in ~0.5 M EDC solution). Prepare EDC immediately before use in Step

B3.

4. Dialysis cassette or desalting column.

B. Procedure

1. Dissolve protein (carboxyl-containing molecule) in MES Buffer at 5-10 mg/mL.
2. Add 25 µL of Hydrazide-Biotin Solution per 1 mL of the protein solution and mix (results in 1.25 mM reagent).
3. Add 12.5 µL of the EDC solution per 1 mL of the protein solution and mix (results in ~6.5 mM EDC).
4. Incubate at 2 hours to overnight at room temperature with mixing.
5. Remove any precipitate that forms during the reaction by centrifugation. Separate the biotinylated molecule from nonreacted material by dialysis or gel filtration (desalting column).

   Note: 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.

Notice

Biotin-Hydrazide can be dissolved in DMSO and then diluted into aqueous reaction mixtures. Do not use DMF, in which reagent solubility is poor. Avoid buffers containing primary amines (e.g., Tris or glycine) or carboxyls (e.g., acetate or citrate) because they will quench the reaction.