Procedure for Protein Labeling with HPDP-Biotin

A. Additional Materials Required

1. Reaction Buffer: Sulfhydryl-free buffer such as phosphate-buffered saline (PBS). Including 1 mM EDTA in the buffer helps maintain reduced sulfhydryls until they have the opportunity to react with the HPDP-Biotin.
2. Solvent: HPDP-Biotin is not soluble in aqueous buffer; it must be dissolved in organic solvent before addition to an aqueous reaction. Use dimethylsulfoxide (DMSO) or dimethylformamide (DMF).
3. (Optional): For separating labeled protein from excess nonreacted HPDP-Biotin: Desalting Columns or Dialysis Cassettes.

B. Material Preparation

HPDP-Biotin Stock Solution: Prepare 4 mM HPDP-Biotin stock solution by adding 2.2 mg HPDP-Biotin to 1.0 mL of Solvent (e.g., DMF). To ensure complete dissolution of the reagent, gently warm the mixture to 37°C and vortex or sonicate. This stock can be aliquoted and stored frozen.

C. Biotinylation of β-D-galactosidase (Protein)

1. Dissolve 2 mg of reduced β-D-galactosidase in 1 mL Reaction Buffer.
2. Add 100 µL of HPDP-Biotin Stock Solution to 1 mL of protein solution (results in 0.4 mM Biotin HPDP).
3. Vortex to mix and then incubate reaction mixture for 2 hours at room temperature.
4. Desalt the reaction mixture using a Desalting Column equilibrated with Reaction Buffer or other suitable storage buffer.

Additional Information

A. Pyridine-2-Thione Assay to Monitor Reaction

1. Immediately before (and/or after) adding HPDP-Biotin to the protein sample, measure and record the absorbance at
343nm of the protein sample compared to a buffer (e.g., PBS) blank.
2. At various time-points after beginning the labeling reaction, measure and record the absorbance at 343nm of the sample.
3. Calculate the change in absorbance: ΔA343 = (Ave. A343 at time-point) – (Ave. A343 at time 0)
4. Calculate the molar ratio of biotin to protein using the following equation:

$$\frac{\Delta A}{8080} \times \frac{\text{MW of Protein}}{\text{mg/ml of Protein}} = \text{moles of HPDP-Biotin reaction (biotinylation) per mole of Protein}$$

Where the value 8080 reflects the extinction coefficient for pyridine-2-thione at 343nm: 8.08 × $10^3 M^{-1} cm^{-1}$

B. Determination of Biotin Incorporation

Biotin incorporation can be estimated using the HABA (4′-hydroxyazobenzene-2-carboxylic acid) method.

Notice

The 2-pyridyldithio group of HPDP-Biotin reacts optimally with free (reduced) sulfhydryls at pH 7-8. Avoid buffers containing thiols or disulfide reducing agents.