Biotin Hydrazide Labeling Reagents

Biotin hydrazides can be used to label glycoproteins, RNA, polysaccharides, or glycolipids. The labeling procedure for glycoproteins, polysaccharides, or glycolipids involves the reaction of the hydrazide with aldehyde groups produced by mild periodate oxidation of cis-diols of carbohydrates. Biotin hydrazides can also be used to biotin-label proteins and peptides by a carbodiimide coupling of the hydrazide to the carboxyl groups of aspartic and glutamic acids. 3' terminal ribose of the RNA can be labeled using biotin hydrazide. Biotin hydrazide will react with the aldehydes formed by periodate oxidation of the cis-diol of 3' terminal ribose.

Protocol for labeling carbohydrate groups on glycoproteins:

1. Dissolve the glycoprotein to be labeled in 100 mM sodium acetate, pH 5.5 at a concentration of 5 mg/ml.
2. Immediately before use, dissolve sodium periodate ($NaIO_4$) in distilled water at a concentration of 100 mM (21.4 mg/ml).
3. Add $NaIO_4$ to the protein solution in the dark to a final concentration of approximately 10 mM in aliquots at 2 minute intervals (e.g. for 1 ml of the sample add 5 x 20 µl of $NaIO_4$ solution at 2 minutes intervals).
4. Incubate in the dark for additional 20 minutes.
5. Separate the oxidized glycoprotein from the $NaIO_4$ using a gel filtration column equilibrated in 100 mM sodium acetate buffer, pH 5.5.
6. Dissolve Biotin (Long Arm) Hydrazide in dimethylsulfoxide at 50 mg/ml and add 40 µl of this solution per one ml of protein solution to be labeled.
7. Incubate 2 hours to overnight at room temperature.
8. Separate the unreacted material from the glycoprotein by gel filtration or dialysis.

Protocol for protein labeling by carbodiimide coupling:

This procedure is designed for labeling carboxyl groups on proteins.

1. Dissolve the protein to be labeled in 150 mM NaCl at 5-10 mg/ml. Insure that the pH of the solution is between pH 5 and pH 6. Adjust, if necessary, with dilute HCl or dilute NaOH.
2. Dissolve biotin hydrazide in dimethylsulfoxide to a concentration of 50 mg/ml.
3. Dissolve 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide-HCI (EDC) in 150 mM NaCl to a concentration of 100 mg/ml.
4. [OPTIONAL] Dissolve N-hydroxysulfosuccinimide (sulfo-NHS) in 150 mM NaCl to a concentration of 10 mg/ml.
   NOTE: the addition of this reagent increases the labeling efficiency but may also result in over-biotinylation of some proteins.
5. Add 20 µl biotin hydrazide, 100 µl EDC and 100 µl sulfo-NHS per one ml of protein solution.
6. Incubate for 3 hours to overnight at room temperature.
7. Separate the unreacted materials from the protein by gel filtration or dialysis.

Protocol for labeling RNA:

The reagent will react with aldehydes formed by mild oxidation of the cis-diol of 3' terminal ribose. Terminal biotinylation with Biotin hydrazide has been used in analysis of mRNA populations in methods like CAGE (Kodzius et al.) or for tRNA immobilization (Shigi et. al.).

1. Dilute the RNA sample to be labeled in 100 mM sodium acetate, pH 4.5 (final concentration of the RNA sample should not be higher than 0.25 mM).
2. Immediately before use, dissolve sodium periodate ($NaIO_4$) in DEPC-treated distilled water at a concentration of 100 mM (21.4 mg/ml).
3. To 90 µl of the RNA sample add 10 µl $NaIO_4$ solution, mix and incubate for 1 hour at 4°C in the dark.

4. Precipitate the oxidized RNA from the reaction by adding 5 µl of 3M sodium acetate pH 5.2 and 0.3 ml of ethanol, mix and centrifuged for 15 minutes at 10,000 x g.
5. Remove supernatant, add 0.3 ml 70% ethanol, mix and centrifuge for 5 minutes at 10,000 x g.
6. Remove supernatant, dry the pellet in vacuum and resuspend in 70 µl DEPC-treated water.
7. Add 10 µl 1M sodium acetate, pH 6.0.
8. Dissolve biotin hydrazide in DMF (dimethyl formamide) at 18.5 mg/ml (the reagent many not dissolve completely but can still be used in the following reaction) and add 20 µl of this solution to the oxydized RNA sample.
9. Mix well and incubate over night at room temperature in the dark.
10. Precipitate the labeled RNA from the reaction by adding 5 µl 3M Na-acetate, pH 5.2, and 0.3 ml ethanol. Centrifuged for 15 minutes at 10,000 xg.
11. Remove supernatant, add 0.3 ml 70% ethanol, mix and centrifuge for 5 minutes at 10,000 xg.
12. Remove supernatant, dry the pellet in vacuum and resuspend in desired volume of DEPC-treated water or buffer.

References:

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