USER GUIDE

Biotin (Long Arm) Hydrazide

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Cat. No.	**SP-1100**
Storage	2-8 °C (desiccated). Once in solution, store frozen.

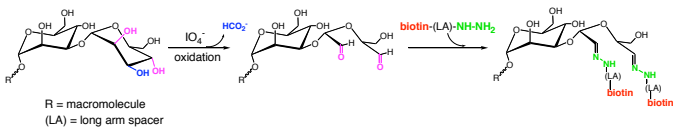
Biotin (Long Arm) Hydrazide can be used to label glycoproteins, RNA, polysaccharides or glycolipids. The labeling procedure for glycoproteins, polysaccharides, or glycolipids involves the reaction of the hydrazide with aldehyde groups produced by mild periodate oxidation of cis-diols of carbohydrates. Biotin (Long Arm) Hydrazide 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.

The 3′ terminal ribose of RNA can be labeled using Biotin (Long Arm) Hydrazide. Biotin (Long Arm) Hydrazide will react with the aldehydes formed by periodate oxidation of the cis-diol of 3′ terminal ribose. Biotin (Long Arm) Hydrazide can also be used to label other biological macromolecules using a method based on generation of a reactive nitrene intermediate.

The presence of the 6-aminohexanoate long-arm spacer between the hydrazide group and the biotin reduces the possibility of steric hindrance and allows the biotin to be fully accessible to streptavidin or avidin conjugates.

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 NaIO4 solution at 2 minute 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. Ensure 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 (Long Arm) Hydrazide in dimethylsulfoxide to a concentration of 50 mg/ml.
3. Dissolve 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide-HCl (EDC) in 150 mM NaCl to a concentration of 100 mg/ml.
4. Dissolve N-hydroxysulfosuccinimide (sulfo-NHS) in 150 mM NaCl to a concentration of 10 mg/ml. (The addition of this reagent increases the labeling efficiency but may also result in over-biotinylation of some proteins.)
5. Add 20 µl Biotin (Long Arm) 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 (Long Arm) 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.

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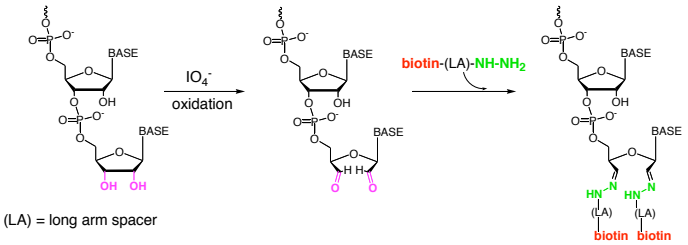
4. Precipitate the oxidized RNA from the reaction by adding 5 µl of 3M sodium acetate pH 5.2 and 0.3 ml of ethanol, mix and centrifuge for 15 minutes at 10,000 x g.
5. Remove supernatant, add 0.3 ml 70% ethanol, mix and centrifuge for 5 minutes at 10,000 x g.
6. Remove supernatant, dry the pellet in vacuum and resuspend in 70 µl DEPC-treated water.
7. Add 10 µl 1M sodium acetate, pH 6.0.
8. Dissolve Biotin (Long Arm) Hydrazide (Cat. No. SP-1100) in dimethyl formamide at 18.5 mg/ml (the reagent may not dissolve completely but can still be used in the following reaction) and add 20 µl of this solution to the oxidized RNA sample.
9. Mix well and incubate overnight 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. Centrifuge for 15 minutes at 10,000 x g.
11. Remove supernatant, add 0.3 ml 70% ethanol, mix and centrifuge for 5 minutes at 10,000 x g.
12. Remove supernatant, dry the pellet in vacuum and resuspend in desired volume of DEPC-treated water or buffer.



Protocol for labeling biological molecules via reactive nitrene intermediate

This procedure can be used to label many proteins and other macromolecules or biological materials.

1. Dissolve the protein to be labeled in a suitable aqueous buffer solution (e.g. PBS, pH 7.9) at 1-5 mg/ml.

2a. Add 2 mg Biotin (Long Arm) Hydrazide to 0.55 ml distilled H^2O; add 0.31 ml of 0.1 N HCl and mix until the reagent is dissolved. Place on ice for 5 minutes.

2b. Dissolve sodium nitrite ($NaNO^2$) in distilled water at a concentration of 10 mg/ml.

2c. Add 80 µl $NaNO^2$ solution to the Biotin (Long Arm) Hydrazide solution. Place on ice for 3 minutes.

3. Add $NaNO^2$ / Biotin (Long Arm) Hydrazide solution dropwise to one ml of the protein solution to be labeled.

4. Incubate in an ice bath 10 cm below a mercury vapor bulb for 15 minutes.

5. Separate the unreacted materials from labeled protein by gel filtration chromatography or dialysis.

References

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