NHS-Biotin/NHS-LC-Biotin

Cat.No.: C100212/C100215
C100212 NHS-Biotin package: 50/250 mg
C100215 NHS-LC-Biotin package: 50 mg

Description

**NHS-Biotin**, N-hydroxysuccinimidobiotin
Molecular Weight: 341.38
Spacer Arm Length: 13.5 Å
Net Mass Added: 226.08
Storage: Upon receipt store desiccated at 4°C. Product is shipped at ambient temperature.

**NHS-LC-Biotin**, succinimidyl-6-(biotinamido)hexanoate
Molecular Weight: 454.54
Spacer Arm Length: 22.4 Å
Net Mass Added: 339.16
Storage: Upon receipt store desiccated at 4°C. Product is shipped at ambient temperature.

Introductions

NHS-Biotin Reagents enable simple and efficient biotin labeling of antibodies, proteins and any other primary amine-containing macromolecules in solution. Differing only in spacer arm lengths, the three reagents offer researchers the possibility of optimizing labeling and detection experiments where steric hindrance of biotin binding is an important factor.

Biotin is a small naturally occurring vitamin that binds with high affinity to avidin and streptavidin proteins. Because it is so small (244 Da), biotin can be conjugated to many proteins without altering their biological activities. Labeled proteins may be purified from unlabeled proteins using immobilized streptavidin and avidin affinity gels, and they may be detected easily in ELISA, dot blot or Western blot applications using streptavidin or avidin-conjugated probes.

N-Hydroxysuccinimide (NHS) esters of biotin are the most popular type of biotinylation reagent. NHS-activated biotins react efficiently with primary amino groups (-NH2) in pH 7-9 buffers to form stable amide bonds. Proteins, including antibodies, generally have several primary amines in the side chain of lysine (K) residues and the N-terminus of each polypeptide that are available as targets for labeling with NHS-activated biotin reagents. Several different NHS esters of biotin are available, with varying properties and spacer arm lengths. The two NHS-Biotin Reagents are not directly water soluble and must be dissolved in organic solvents such as DMSO or DMF before addition to aqueous solutions at the final concentration for the labeling reaction.

![Figure](image-url). Reaction of NHS-LC-Biotin with primary amine
Biotinylation of intact cells has emerged as an important tool for studying the expression and regulation of receptors and transporters, differentiation of plasma membrane proteins from those localized to organelle membranes, and distribution of membrane proteins in polarized epithelial cells. Sulfo-NHS-Biotin reagents (see Related Thermo Scientific Products) do not readily permeate cell membranes and are commonly used for specifically labeling the cell surface. By contrast, NHS-Biotin reagents are membrane permeable and may be used to biotinylate proteins inside intact cells. Parallel experiments with NHS-and Sulfo-NHS-Biotin analogs may help to localize particular proteins of interest.

**Additional Materials Required**

1. Water-miscible organic solvent such as dimethylsulfoxide (DMSO) or dimethylformamide (DMF)
2. Phosphate Buffered Saline (PBS) or other amine-free buffer having pH 7-8 for use as reaction buffer (see Important Product Information and Related Thermo Scientific Products)
3. Desalting columns or dialysis units for buffer exchange (see Important Product Information and Related Thermo Scientific Products)

**Procedure for Biotinylating Proteins**

The following procedure ordinarily will yield incorporation of 3-5 biotins per molecule of protein. Antibodies, which are large proteins, often will label with ~8-12 biotin molecules per molecule of IgG, especially when greater molar excesses of biotin reagent are used. The molar ratio of biotin reagent to protein may be adjusted to obtain the level of incorporation desired.

**A. Calculations**

The amount of biotin reagent to use for each reaction depends on the amount of protein to be labeled and its concentration. By using the appropriate molar ratio of biotin to protein, the extent of labeling can be controlled. When labeling more dilute protein solutions, a greater molar fold excess of biotin is necessary to achieve the same results. Generally, for best results use 12-fold molar excess of biotin for a 10 mg/mL protein solution or 20-fold molar excess of biotin for a 2 mg/mL protein solution.

**B. Biotin Labeling Reaction**

1. If the biotin reagent has been stored cold, remove the vial from storage and fully equilibrate it to room temperature before opening in step 3.
2. Dissolve 1-10 mg protein in 0.5-2.0 mL PBS according to the calculation made in section A.
   - Protein that is already dissolved in amine-free buffer at pH 7.2-8.0 may be used without buffer exchange or dilution with PBS. Proteins in Tris or other amine-containing buffers must be exchanged into a suitable buffer.
3. Immediately before use, prepare a 10 mM solution of the biotin reagent in an organic solvent such as dimethylsulfoxide (DMSO) or dimethylformamide (DMF):
   - For NHS-Biotin, dissolve 2.0 mg reagent in 590 μl of solvent.
   - For NHS-LC-Biotin, dissolve 2.3 mg reagent in 500 μl of solvent.
4. Add the appropriate volume (see Calculations in section A) of 10 mM biotin reagent solution to the protein solution.
5. Incubate reaction on ice for two hours or at room temperature for 30 minutes.
   - Other than the possibility of ordinary protein degradation or microbial growth, there is no harm in reacting longer than the specified time.
6. Protein labeling is complete at this point, and although excess non-reacted and hydrolyzed biotin reagent remains in the solution, it is often possible to perform preliminary tests of the labeled protein by ELISA or Western blot. Once proper function and labeling of the protein has been confirmed, the labeled protein may be purified for optimal performance and stability using desalting or dialysis. If the level of biotin incorporation will be determined using the Biotin Quantitation Kit (HABA assay; see Related Thermo Scientific Products), the protein first must be desalted or
dialyzed to remove non-reacted biotin.

**Procedure for Biotinylating Cells**

Many variations of this procedure exist in the literature. Sulfo-NHS-Biotin reagents (see Related Thermo Scientific Products) do not readily permeate cell membranes and are commonly used for specifically labeling the cell surface. By contrast, NHS-Biotin reagents are membrane permeable and may be used to biotinylate proteins inside intact cells. Parallel experiments with NHS- and Sulfo-NHS-Biotin analogs may help to localize particular proteins of interest.

Labeling may be performed on cells in suspension or on adherent cells in culture plates. In the latter situation, diffusion of the NHS-Biotin reagent to all surfaces of the cells will be limited, and labeling will occur predominately on and through the exposed surface. Culture media must be washed from the cells, or amine-containing components will compete and quench the reaction to cell proteins. Using a more concentrated cell suspension is most effective since less biotin reagent will be required in the reaction. Generally, a final concentration of 2-5 mM NHS-Biotin reagent is effective. NHS-Biotin reactions occur more rapidly at higher pH; therefore, pH 8.0 is used in the following example so that labeling can be completed as quickly as possible.

1. Wash cells three times with ice-cold PBS (pH 8.0) to remove amine-containing culture media and proteins from the cells.
2. Suspend cells at a concentration of ~25 x10⁶ cells/mL in PBS (pH 8.0).
3. Prepare a 20 mM solution of NHS-Biotin reagent by dissolving 4-5 mg of reagent per 0.5 mL of water-miscible organic solvent such as dimethylsulfoxide (DMSO) or dimethylformamide (DMF).
4. Add 100 μl of NHS-Biotin reagent solution to each 1 mL of cell suspension (results in ~2 mM biotin reagent).
5. Incubate reaction mixture at room temperature for 30 minutes.
6. Wash cells three times with PBS + 100 mM glycine to quench and remove excess biotin reagent and byproducts.
7. Lyse and/or analyze biotin-labeled cells as required for the research method.

**Note**

1. NHS-Biotin reagents are moisture-sensitive. If the vial of reagent has been stored cold, fully equilibrate vial to room temperature before opening to avoid moisture condensation inside the container.
2. As directed in the procedure, dissolve the biotin reagent immediately before use. The NHS ester moiety readily hydrolyzes and becomes nonreactive; therefore, weigh and dissolve only a small amount of the reagent at a time, and do not prepare stock solutions for storage. Discard any unused reconstituted reagent.
3. Avoid buffers containing primary amines (e.g., Tris or glycine) as these will compete with the reaction (see Figure 1). If necessary, dialyze or otherwise desalt to exchange the protein sample into an amine-free buffer such as phosphate buffered saline (PBS).
4. When biotinylating proteins in solution, excess non-reacted biotin and reaction byproducts are easily removed by size exclusion using either desalting columns or dialysis. A 10 mL desalting column is best suited for processing biotinylation reactions involving 1-10 mg of protein in approximately 0.5-2 mL. For smaller amounts of protein and/or smaller reaction volumes, both the biotinylation reaction and subsequent buffer exchange may be performed in a single MINI Dialysis Unit. For larger reaction volumes than can be processed with a desalting column, either split the sample between two columns or use an appropriate Dialysis Cassette for buffer exchange steps.