Lumiprobe
Life science solutions

NHS Ester Labeling of Biomolecules Containing Primary Amino Groups

NHS (N-HydroxySuccinimide) esters and other activated esters (sulfo-NHS, sulfotetrafluorophenyl — STP) are reactive compounds suitable for the modification of amino groups. NHS is the most common type of activated esters.

Usual modifications are fluorescent labels, fluorescence quenchers, and other reporter groups. Alkyne and azido groups can be attached using activated esters to adapt biomolecules to click chemistry.

Especially common are modifications of proteins and peptides since they nearly always contain amino groups. Other examples are modifications of amino-oligonucleotides, amino-modified DNA, and amino-containing sugars.

The reaction of NHS esters with amino groups is strongly pH-dependent: at low pH, the amino group is protonated, and the modification does not occur. At higher-than-optimal pH, hydrolysis of NHS ester is quick, and the yield of modified molecule decreases. The optimal pH value for modification is 8.3-8.5.

Water is the most common solvent used to dissolve NHS esters for biomolecule labeling. If NHS ester is poorly soluble in aqueous solutions, it can be at first dissolved in dimethyl sulfoxide (DMSO) or dimethylformamide (DMF) and then added to a solution of protein in a buffer with pH 8.3-8.5.

Important! Note that DMF can degrade into dimethylamine, which has a fishy smell and is able to react with activated esters. Therefore, to label biomolecules, it is important to use high-quality DMF that contains no dimethylamine and has no fishy odor.

We recommend using the following general protocol for the labeling of biomolecules with NHS esters produced by Lumiprobe:

1. Calculate the required amount of NHS ester using the formula below or our protein labeling calculator:

   NHS_ester_weight [mg] = 8 × amino_compound_weight [mg] × NHS_ester_molar_weight [Da] / amino_compound_molar_weight [Da].

   8 is a molar excess of NHS ester. It is an empirical value for mono-labeling, suitable for many common proteins and peptides. However, in some cases using less or more NHS ester can increase the yield of the modified biomolecule. It depends on protein structure, reagent used for labeling, and its solubility. The molar weight of Lumiprobe products can be found on the corresponding product pages.

   For example, to label 3 mg of BSA (molar weight 66500 Dalton) with Cy5 NHS ester (molar weight 616 Dalton), and obtain maximum yield of mono-labeled product, one should use 8 × 3 mg × 616 Da / 66500 Da = 0.22 mg of Cy5 dye NHS ester.

2. Calculate the volume of the reaction mixture. The optimal biomolecule concentration in labeling reaction is 1-10 mg per mL. The labeling can be performed on any scale, from nanomols to dozens of grams. If you label a low amount of biomolecule, keep the volume of the reaction mixture at a minimum (10-20 uL).
3. Dissolve NHS ester in 1/10 reaction volume of water, DMF, or DMSO. Dimethylamine-free DMF or water are preferred solvents. NHS ester dissolved in DMF can be stored in solution for 1-2 months at -20°C. The aqueous solution of NHS ester should be used immediately after preparation.
4. Dissolve biomolecule in 9/10 reaction volume of buffer with pH 8.3-8.5.

Lumiprobe Corporation
115 Airport Dr Suite 160
Westminster, Maryland 21157
USA
Phone: +1 888 973 6353
Fax: +1 888 973 6354
Email: order@lumiprobe.com

Lumiprobe GmbH
Feodor-Lynen-Strasse 23
30625 Hannover
Germany
Phone: +49 511 16596811
Fax: +49 511 16596815
Email: de@lumiprobe.com

Lumiprobe RUS Ltd
Kotsyubinsky street, 4
121351 Moscow
Russian Federation
Phone: +7 800 775 3271
Email: ru@lumiprobe.com

Lumiprobe Limited
Suite 12, 3/F, Great Eagle Centre
23 Harbour Road, Wan Chai
Hong Kong
Mob.: +852-5929-0488 (from HK)
Phone: +86-147-14316277 (from China)
Email: hk@lumiprobe.com

Lumiprobe LTD
2 Tuvim St.
3223562, Haifa
Israel
Phone: +972-(0)4-374-0377
Email: il@lumiprobe.com

Lumiprobe Co., Ltd.
10H-11, Shenmao Commercial Center
No. 59 Xinwen Rd., Meiling Community
Lianhua Street, Futian District
Shenzhen, China
Phone: +86-1471431-6277
Email: cn@lumiprobe.com

0.1 M Sodium bicarbonate solution has appropriate pH. Another alternative is 0.1 M phosphate buffer. Note that pH is the most important factor for successfully labeling biomolecule with NHS ester. Avoid buffers containing amines (Tris-based buffers can sometimes be used but are not recommended. Tris contains amino group, but its affinity to activated esters is low).

When doing large-scale labeling (using hundreds of milligrams of NHS ester), note that the mixture tends to acidify with time because of the hydrolysis of NHS ester. Monitor pH, or use a more concentrated buffer then.

5. Add NHS ester solution to the solution of biomolecule, and vortex well. Keep on ice overnight, or at room temperature from 1 to 4 hours.
6. Purify the conjugate using the appropriate method: gel-filtration for macromolecules is the most common. Chromatography is another alternative. For proteins and nucleic acids, ethanol or acetone precipitation can be used. Organic impurities (such as N-hydroxysuccinimide, NHS ester, and acid produced by hydrolysis) are almost always easily separated by these methods.

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Lumiprobe GmbH
Feodor-Lynen-Strasse 23
30625 Hannover
Germany
Phone: +49 511 16596811
Fax: +49 511 16596815
Email: de@lumiprobe.com

Lumiprobe RUS Ltd
Kotsyubinsky street, 4
121351 Moscow
Russian Federation
Phone: +7 800 775 3271
Email: ru@lumiprobe.com

Lumiprobe Limited
Suite 12, 3/F, Great Eagle Centre
23 Harbour Road, Wan Chai
Hong Kong
Mob.: +852-5929-0488 (from HK)
Phone: +86-147-14316277 (from China)
Email: hk@lumiprobe.com

Lumiprobe LTD
2 Tuvim St.
3223562, Haifa
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Phone: +972-(0)4-374-0377
Email: il@lumiprobe.com

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10H-11, Shenmao Commercial Center
No. 59 Xinwen Rd., Meiling Community
Lianhua Street, Futian District
Shenzhen, China
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Email: cn@lumiprobe.com