TECHNOTE 106
NHS 的介绍及其使用

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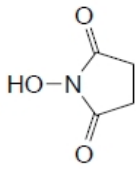
中文名：N-羟基琥珀酰亚胺
英文名： *N*- 

hydroxysuccinimide
简称：NHS
CAS: 6066-82-6
分子量: 115.1
描述：可用于合成氨基酸保护剂，常和碳二亚胺连用做交联剂，羧酸（-COOH）在 EDC 的存在下与 NHS 反应得到半稳定的 NHS 酯，然后与伯胺（-NH2）反应形成酰胺键。尽管不需要 NHS 进行碳二亚胺的反应，但是 NHS 的使用大大提高了偶联的效率，也同时提供了另外一个偶联的方式（两步法）。

除了常用的 NHS 可以做为碳二亚胺的一个稳定剂之外，还有一个物质 Sulfo-NHS (N-hydroxysulfosuccinimide, CAS# 106627-54-7)也可以同 NHS 一样使用。较 NHS 而言，Sulfo-NHS 所形成的半稳定中间体水溶性更好。NHS 酯和 Sulfo-NHS 酯在 pH=7 时的半衰期为 4 小时，在 pH=8 时的半衰期为 1 小时，在 pH=8.5 时的半衰期为 10 分钟，这也为两步活化法提供了依据。

NHS 和 Sulfo-NHS 在 pH4.5~7.2 之间活性最高，而 EDC 经常在 MES Buffer 的 pH 范围为 4.5~6，在和伯胺反应时 pH 优选的范围为 7~8，所以采用两步法反应时，先在 MES Buffer（pH 5-6）活化，在 PBS Buffer(pH 7~8)中进行氨基反应。
形状：白色或类白色结晶粉末
溶解度：溶于水，易溶于丙酮、醇、乙酸乙酯，微溶于醚、甲苯、苯。对潮湿敏感，易结块。
保存温度：-4℃
注意事项：NHS 在 280 处具有强烈的吸收，在做蛋白质偶联效率测定的时候，注意影响。

Procedure for EDC/NHS Crosslinking of Carboxylates with Primary Amines

A：Materials Required

□□ Activation Buffer: 0.1 M MES (2-[morpholino]ethanesulfonic acid), 0.5 M NaCl, pH 6.0.

□ Phosphate-buffered Saline (PBS): 0.1 M sodium phosphate, 0.15 M NaCl, pH 7.2-7.5

□□ Protein: Prepare 1 ml of Protein in activation buffer at ~10 mg/ml

□□ Hapten: dissolved at 1-10 mg/ml in PBS or other amine-free buffer, pH 7-8
□□ EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide)

B. NHS-ester Activation

1. Add 0.4 mg of EDC (final concentration 2 mM) directly to 1 ml of Protein, which based on a 50 kDa protein, results in a 10-fold molar excess of EDC to Protein.
2. Add either 0.6 mg of NHS or 1.1 mg of Sulfo-NHS to the reaction (final concentration 5 mM).
3. Mix reaction components well and react for 15 minutes at room temperature.
4. (Optional): Separate activated Protein from excess EDC, EDC-byproducts, NHS and (if used) 2-mercaptoethanol using an appropriate size desalting column that has been equilibrated with PBS. Follow desalting column instructions and recover the fraction containing the activated protein. If using absorbance at 280 nm to identify fractions containing protein, be aware that NHS and Sulfo-NHS absorb strongly at 260-280 nm.

C. Amine Reaction

1. If step B.4 was not performed (i.e., buffer not exchanged using a desalting column), then increase buffer pH above 7.0 using concentrated PBS or other non-amine buffer such as sodium bicarbonate.
2. Add Hapten to the solution containing activated Protein.
3. Mix the solution well and then allow reaction to proceed for 2 hours at room temperature.
4. (Optional): Quench reaction by adding hydroxylamine to a final concentration of 10 mM. The excess hydroxylamine reacts to all NHS esters remaining on the surface of Protein, resulting in conversion of the original carboxyl groups to a hydroxamic acid. Alternative quenching reagents include 20-50 mM Tris, lysine, glycine and ethanolamine. Addition of base to raise the pH > 8 will promote hydrolysis of the NHS esters, thereby regenerating the original carboxyl groups.

Schematic diagram