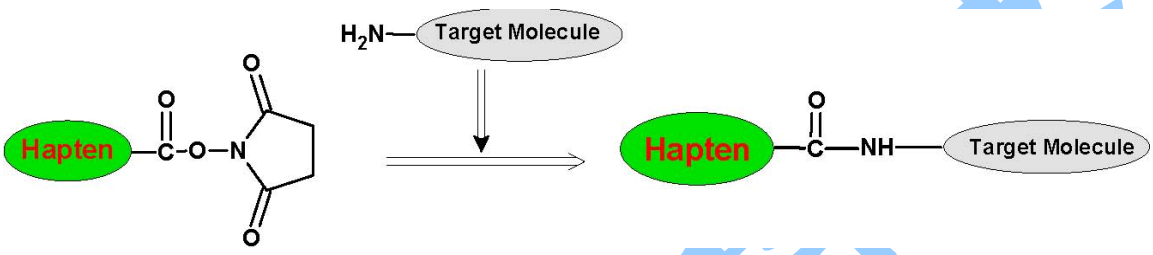
MTW生物免疫标记荧光染料NHS酯

1. 标记原理

NHS esters are proven to be the best reagents for amine modifications because the amide bonds that are formed are essentially identical to, and as stable as the natural peptide bonds. These reagents are generally stable and show good reactivity and selectivity with aliphatic amines.



2. 影响因素

There are few factors that need be considered when NHS ester compounds are used for conjugation reaction:

1). Solvents(溶剂):

For the most part, reactive dyes are hydrophobic molecules and should be dissolved in anhydrous dimethylformamide (DMF) or dimethylsulfoxide (DMSO).

2). Reaction pH（pH值）:

The labeling reactions of amines with succinimidyl esters are strongly pH dependent. Amine-reactive reagents react with non-protonated aliphatic amine groups, including the terminal amines of proteins and the ε-amino groups of lysines. Thus amine acylation reactions are usually carried out above pH 7.5. Protein modifications by succinimidyl esters can typically be done at pH 7.5-8.5, whereas isothiocyanates may require a pH 9.0-~10.0 for optimal conjugations.

3). Reaction Buffers（缓冲溶液）:

Buffers that contain free amines such as Tris and glycine and thiol compounds must be avoided when using an amine-reactive reagent. Ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation must also be removed (such as viadinlysis) before performing dye conjugations.

4). Reaction Temperature（反应温度）：

Most conjugations are done at room temperature. However, either elevated or reduced temperature may be required for a particular labeling reaction.

3. 标记方法（参考）

3.1. NHS-ester Dyes Coupling protocol for proteins

1） To prepare a stock solution of the label, dissolve 1 mg of the NHS-ester Dyes in 50 µl absolute, amine-free DMF (final concentration: approx. 25 nmol·µl-1).

2） Dissolve the desired amount of protein in bicarbonate buffer (pH 9.0, 50 mM).

3） Transfer an appropriate volume of the NHS-ester Dyes stock solution dropwise under stirring to the protein solution. Due to the high reactivity of the NHS-ester Dyes add an equimolar amount or the double excess of label to the protein to obtain a dye to protein ratio (D/P) between 1 and 2. Higher molar excesses of the label can lead to overlabeling of the protein causing a decrease in quantum yield of the conjugate.

4） Let the mixture react for one hour at room temperature.

5） Separate the obtained protein conjugate from unreacted free dye using a Sephadex column (Sephadex G25 medium; eluent PBS pH 7.2, 22 mM). Normally, the first running coloured band is the Dye labeled protein.

3.2. NHS-ester Dyes Coupling protocol for Antibodies

1） Antibodies to be conjugated should at a minimum be purified over a Protein A- or Protein G-Sepharose column prior to any conjugation reactions. Affinity-purified antibodies are ideal. Antibodies should be at an approximate protein concentration of

at least 1 mg/ml total protein for conjugation. Total volume of antibodies should be between 0.5 and 2 mls. Larger volumes of antibodies often show reduced amine modification.

2） Dialyze the antibody prep against 0.1 M sodium bicarbonate buffer pH 8.5 for at least 4 hours prior to conjugation.

3） Dissolve the biotin- or flurochrome-NHS ester at 1 mg/ml in dry DMSO. All NHS-ester solutions should be made immediately prior to the conjugation process, as solutions of these compounds will gradually hydrolyze in solution. Use high-grade DMSO (water-free) - DMSO stored over molecular sieve to prevent water accumulation is recommended.

4） Remove the antibody from dialysis and transfer to a 1.5 ml Eppendorf tube. With constant gentle vortexing, slowly add 125 microliters of the NHS ester solution to every 1 ml of antibody. This constitutes an 8:1 w/w ratio of antibody to biotin or fluorochrome. This ratio has been empirically determined for a variety of human and rodent antibodies and is a good starting point for determining the optimal protein:fluorochrome ratio. Ratios from 4:1 to 10:1 may need to be tested to obtain the optimal fluorochrome:proteins ratio.

5） Incubate at room temperature for four hours with periodic mixing.

6） Transfer the reaction mixture to dialysis tubing and dialyze against three 1000-fold volumes of PBS pH 7.4 at 4 ^{o}C to completely remove unreacted biotin or fluorochrome. Alternately, the antibody can be separated on a column (if the fluorochrome is chromogenic). Normally, the fluorochrome-antibody conjugate will elute first.

7） Determine the ratio of protein to fluorochrome spectrophotometrically. For **fluorescein conjugations**, this can be done using a spectrophotometer. Measure the absorbance of the conjugate at 280 nm (the protein concentration) and 495nm (the fluorescein concentration).

1 mg/ml IgG = absorbance of 1.4 at 280 nm

1 mM fluorescein = 68 at 495 nm and 11.9 at 280 nm

For fluorescein-conjugated antibodies, the protein concentration equals...

IgG (mg/ml) = [A(280) - 0.31 * A(495)]/1.4]

Then calculate the F/P ratio...

For IgG: 3.1 * A(495)/[A(280) - 0.31 * A (495)]

Good conjugations can range from an F/P ratio of 3 to 10.

4. 公司产品（荧光标记染料）：

货号	荧光染料 NHS 酯
MTW-F001	5-羧基四甲基罗丹明 NHS 酯（单一化合物）
MTW-F002	6-羧基四甲基罗丹明 NHS 酯（单一化合物）
MTW-F003	5(6)-羧基四甲基罗丹明 NHS 酯(混合物)
MTW-F004	5-羧基-X-罗丹明 NHS 酯(单一化合物)
MTW-F005	6-羧基-X-罗丹明 NHS 酯(单一化合物)
MTW-F006	Cy3-N-羟基 NHS 酯
MTW-F007	Cy5-N-羟基 NHS 酯
MTW-F008	Cy7-N-羟基 NHS 酯
MTW-F009	5-羧基荧光素 NHS 酯(单一化合物)
MTW-F010	6-羧基荧光素 NHS 酯(单一化合物)
MTW-F011	5(6)-羧基荧光素 NHS 酯（混合物）
MTW-F012	6-羧基-2',4,4',5',7,7'-六氯荧光素 NHS 酯
MTW-F013	6-羧基-4',5'-二氯-2',7'-二甲氧基荧光素 NHS 酯

MTW-F014	5(6)-羧基-X-罗丹明 NHS 酯（混合物）
MTW-F015	6-羧基-2',4,7,7'-四氯荧光素 NHS 酯

5. 联系方式

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开户行行号：105290081045

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