AT dyes NHS Ester

Cat #: B-CHM502 / B-CHM503 / B-CHM504 / B-CHM505

Size: 1 mg

Storage: Store at -20°C protected from light.

Product specifications

Catalog No.	Dye-NHS	Ex/Em(nm)	MW	M^+	Δm	Δq	εmax	CF260	CF280
B-CHM502	AT 550 NHS	554nm/576nm	791	691	576.8	+1	120000	0.23	0.1
B-CHM503	AT 565 NHS	564nm/590nm	708	608	492.6	0	120000	0.27	0.12
B-CHM504	AT 590 NHS	593nm/622nm	788	688	572.7	0	120000	0.39	0.43
B-CHM505	AT 655 NHS	663nm/680nm	887	625	509.6	0	125000	0.24	0.08

MW: molecular weight of the dye including counterions in g/mol; M+: molecular weight of dye cation (HPLC_MS acetonitrile/water 0.1 vol-% trifluoroacetic acid); Δm: increase of molecular mass on conjugation with AT-dye NHS-ester; Δq: increase of electrical charge on conjugation with AT-dye NHS-ester; εmax: molar decadic extinction coefficient at the longest-wavelength absorption maximum in M-1 cm-1; CF260 = ε260ε/max; CF280 = ε280ε/max

Product Introduction

AT NHS esters readily react with the amino groups of proteins. We can offer a variety of high-quality amine-reactive dyes for labeling proteins and other amine-containing substrates. The dyes cover a spectral range from 350 nm in the ultraviolet to 750 nm in the near-infrared.

The most commonly used amine-reactive reagent is N-hydroxysuccinimide (NHS) ester. NHS esters readily react with compounds containing amino groups to form chemically stable amide bonds between the dye and the protein. However, the amino groups must be unprotonated to be active. Therefore, the pH of the solution must be adjusted to a sufficiently high level to obtain a high concentration of unprotonated amino groups. On the other hand, NHS esters can

also react with hydroxide ions in the solution, leading to the formation of "free" dyes that are no longer active. Due to the inevitable hydrolysis rate, which increases with the concentration of hydroxide ions, the pH should be kept as low as possible. A buffered solution at pH 8.3 has been found to be a good compromise between these conflicting requirements.

Product Properties

Form: Crystalline solid

Spectral properties: See above

Shipping and Storage

Storage conditions: Store at -20°C protected from light.

Stability: Stable for at least 12 months under proper storage conditions.

Suggested Protocol For Labeling Proteins with AT NHS

AT NHS esters readily react with the amino groups of proteins. The optimal pH range for NHS ester conjugation is pH 8.0-9.0. At this pH range, the amino groups of proteins, specifically the amino groups of lysine, are sufficiently deprotonated for rapid coupling.

Required Materials:

- **Solution A**: PBS buffer (phosphate-buffered saline, pH 7.4): Dissolve 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na2HPO4 - 2H2O, and 0.24 g of KH2PO4 in 1L of distilled water.
- **Solution B**: 0.2 M sodium bicarbonate solution, adjust the pH to 9.0 using 2 M sodium hydroxide.
- **Solution C**: Mix 20 parts of Solution A with 1 part of Solution B to obtain a labeling buffer with a pH of 8.3. Store this solution in a sealed bottle for long-term stability.
- **Solution D**: Dissolve 1.0 mg of the NHS ester in 50-200 ul of anhydrous, amine-free dimethyl sulfoxide (DMSO) or acetonitrile.

Preparation and Handling of Dye Stock Solution

When determining the concentration of the dye stock solution, we suggest taking an equal amount of the sample and diluting it with acidified ethanol (0.1 vol.-% trifluoroacetic acid) to avoid dye aggregation and, in some cases (AT 565 and AT 590), the formation of colorless lactones.Depending on the quality of the solvent, such stock solutions are not stable at room temperature and must be stored light-protected at -20 °C.

Active molecules can undergo hydrolysis and lose their activity. We recommend freshly preparing the dye stock solution immediately before starting the labeling reaction whenever possible. It should be noted that solvents such as DMSO or DMF should be free of nucleophilic and/or alkaline impurities. These compounds can react with the NHS ester functional group, reducing the coupling efficiency.

Conjugate Preparation

The protein solution can not contain any amine-containing substances such as Tris, free amino acids, or ammonium ions. Antibodies dissolved in amine buffer should be dialyzed against Solution A and the desired coupling pH of 8.3 achieved through the steps described in Solution C.

• To achieve an average degree of labeling (DOL) of 2-3 (the ratio of dye to protein), gently add a three-fold molar excess of the active dye (Solution D) to the protein solution while gently shaking. Variations may occur due to differences in reactivity between the protein and labeling reagent. It is necessary to optimize the ratio of dye to protein during the reaction to achieve the desired DOL. Higher ratios of NHS ester to protein must be used to increase the labeling efficiency, and vice versa.

• Incubate the reaction mixture at room temperature, protected from light, for 1 hour. For AT565-NHS and AT590-NHS, we recommend an incubation time of 18 hours at ambient temperature to complete the reaction.

Conjugate Purification - Removal of Unbound Dye

• Due to inevitable side reactions, some NHS esters hydrolyze during the labeling reaction and must be removed from the protein conjugate. We recommend using a Sephadex G-25 (or equivalent) gel filtration column with a diameter of 1-2 cm and a length of 10-20 cm. For highly hydrophilic dyes such as AT488, AT532, AT542, AT594, and AT643, the column length should be at least 30 cm for satisfactory results.

• Equilibrate the column with Solution A.

• Elute the dye-protein conjugate using Solution A.

• The first fluorescent region to elute contains the desired dye-protein conjugate. The second fluorescent region, which moves more slowly, contains unbound free dye (hydrolyzed NHS ester).

• To prevent denaturation of the conjugate after elution, bovine serum albumin (BSA) or other stabilizers can be added.

• To reuse the Sephadex column, elute with a 0.01% sodium hydroxide solution and/or a 80:20 water/ethanol solution to remove residual dye or dye conjugates. Rinse thoroughly with water after treatment.

Storage of Protein Conjugates

In general, the storage conditions for conjugates should be the same as those for the unmodified protein. When storing the solution at 4°C, it is advisable to add sodium azide as a preservative (final concentration of 2 mM). In applications where the conjugate is added to live cell specimens, it may be necessary to remove the preservative before use to avoid inhibitory effects. Conjugates should be stable for several months at 4°C. For long-term storage, the solution can be divided into aliquots and frozen at -20°C. Avoid repeated freezing and thawing. Dye conjugates should be stored light-protected whenever possible.

Determining the Average Degree of Labeling (DOL)

The average degree of labeling (DOL), which represents the ratio of dye molecules to protein molecules, can be determined using the Lambert-Beer law through absorbance spectroscopy: Absorbance (A) = Extinction coefficient (ε) × molar concentration × path length (d). Simply measure the UV-visible spectrum of the conjugate solution after gel filtration in a quartz (UV-transparent) cuvette. If the solution concentration is too high for accurate absorbance measurement, you may need to dilute the solution. Measure the absorbance (A_{max}) at the maximum absorbance wavelength (abs) of the dye and at 280 nm (A280) (maximum absorbance of the protein). The calculation formula for dye concentration is: c(dye) = A_{max} / ε_{max} × d, where ε_{max} is the extinction coefficient of the dye at the maximum absorbance wavelength. Similarly, determine the protein concentration based on the absorbance at 280 nm using the formula: c(protein) = A_{prot} / ε_{prot} × d, where ε_{prot} is the extinction coefficient of the protein at 280 nm. Since all dyes have some absorbance at 280 nm, the measured absorbance at A280 must be corrected for the contribution of the dye. The protein concentration can be calculated as: $A_{prot} = A_{280} - A_{max} \times CF_{280,}$ and then c(protein) = A_{prot} / ε_{prot} × d. The degree of labeling, representing the average number of dye molecules conjugated to protein molecules, can be determined based on the above relationships.

$$DOL = \frac{c(dye)}{c(protein)} = \frac{A_{max}/\varepsilon_{max}}{A_{prot}/\varepsilon_{prot}} = \frac{A_{max} \cdot \varepsilon_{prot}}{(A_{280} - A_{max} \cdot CF_{280}) \cdot \varepsilon_{max}}$$

Note:

The product listed herein is for research use only and is not intended for use in human or clinical diagnosis.