XFD532 NHS Ester *Same Structure to Alexa Fluor™ 532 NHS Ester*

Catalog number: 1819, 71904
Unit size: 1 mg, 5 mg

Component	Storage	Amount (Cat No. 1819)	Amount (Cat No. 71904)
XFD532 NHS Ester	Freeze (< -15 °C), Minimize light exposure	1 mg	5 mg

OVERVIEW

XFD532, manufactured by AAT Bioquest, is structurally identical to Alexa Fluor™ 532 (ThermoFisher). It is a bright yellow-fluorescent dye with an excitation optimized for use with the 532 nm line of the frequency-doubled Nd:YAG laser. XFD532 demonstrates good aqueous solubility and pH-insensitivity over a broad pH range (pH 4–10), ensuring stable fluorescence generation under varying experimental conditions. This dye is particularly suited for multicolor fluorescence microscopy and flow cytometry, as well as advanced applications in super-resolution imaging techniques such as dSTORM.

The N-hydroxysuccinimidyl (NHS) ester of XFD532 is a widely used reagent for the conjugation of this dye to proteins or antibodies. NHS esters react selectively and efficiently with primary amines (such as the side chains of lysine residues or aminosilane-coated surfaces) at pH 7-9, forming stable covalent amide bonds. This property makes XFD532 NHS ester an excellent choice for labeling proteins, amine-modified oligonucleotides, and other amine-containing molecules.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Protein Stock Solution (Solution A)

1. Prepare a 1 mL protein labeling stock solution by mixing 100 µL of reaction buffer (such as 1 M sodium carbonate solution or 1 M phosphate buffer, pH ~9.0) with 900 µL of the target protein solution (e.g., an antibody with a protein concentration of at least 2 mg/mL, if possible).

   Note: The pH of the protein solution (Solution A) should be 8.5 ± 0.5. If the pH of the protein solution is lower than 8.0, adjust it to within the 8.0-9.0 range using either 1 M sodium bicarbonate solution or 1 M phosphate buffer at pH 9.0.

   Note: The protein should be dissolved in 1X phosphate-buffered saline (PBS), pH 7.2-7.4. If the protein is dissolved in Tris or glycine buffer, dialyze it against 1X PBS, pH 7.2-7.4, to remove any free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) commonly used in protein precipitation.

   Note: Antibodies that are impure or stabilized with bovine serum albumin (BSA) or gelatin may not label effectively. Additionally, sodium azide or thimerosal can interfere with the conjugation reaction. To achieve optimal labeling results, these preservatives should be removed through dialysis or spin column techniques.

   Note: For optimal labeling efficiency, it is recommended to maintain a final protein concentration between 2-10 mg/mL. Protein concentrations below 2 mg/mL can significantly reduce conjugation efficiency.

XFD532 NHS Ester Stock Solution (Solution B)

1. To prepare a 10 mM stock solution of XFD532 NHS ester, add anhydrous DMSO directly to the vial of XFD532 NHS ester. Mix well by pipetting or vortexing.

   Note: Prepare the dye stock solution (Solution B) before starting the conjugation, and use it promptly. Extended storage of the dye stock solution may reduce the dye activity. Solution B can be stored in the freezer for up to two weeks, provided it is protected from light and moisture. Avoid freeze-thaw cycles.

SAMPLE EXPERIMENTAL PROTOCOL

This protocol is designed for labeling Goat anti-mouse IgG with XFD532 NHS ester. Additional optimization may be required to adapt the protocol to your specific proteins.

Note: Each protein requires a distinct dye/protein ratio, which varies depending on the characteristics of the dye. Over-labeling a protein can negatively impact its binding affinity, whereas using a low dye-to-protein ratio in protein conjugates can result in reduced sensitivity.

Run Conjugation Reaction

1. Use a 10:1 molar ratio of Solution B (dye) to Solution A (protein) as the starting point: Add 5 µL of the dye stock solution (Solution B, assuming the dye stock solution is 10 mM) to the vial containing the protein solution (95 µL of Solution A) with effective shaking. The concentration of the protein is ~0.05 mM, assuming the protein concentration is 10 mg/mL and the molecular weight of the protein is ~200KD.

   Note: We recommend using a 10:1 molar ratio of Solution B (dye)/Solution A (protein). If it is too low or too high, determine the optimal dye/protein ratio at 5:1, 15:1, and 20:1, respectively.

2. Continue to rotate or shake the reaction mixture at room temperature for 30-60 minutes.

Purify the Conjugate

The following protocol demonstrates the purification of a dye-protein conjugate using a Sephadex G-25 column.

1. Prepare the Sephadex G-25 column according to the manufacturer's instructions.

2. Carefully load the reaction mixture (from the "Run Conjugation Reaction" step) to the top of the Sephadex G-25 column.

3. Add PBS (pH 7.2-7.4) as soon as the sample runs just below the top resin surface.

4. Add more PBS (pH 7.2-7.4) to the desired sample to complete the column purification. Combine the fractions that contain the desired dye-protein conjugate.

   Note: For immediate use, the dye-protein conjugate must be

diluted with staining buffer, and aliquoted for multiple uses.

Note: For longer-term storage, the dye-protein conjugate solution needs to be concentrated or freeze-dried.

Characterize the Desired Dye-Protein Conjugate

The Degree of Substitution (DOS) is a critical factor in characterizing dye-labeled proteins. Proteins with a lower DOS generally exhibit weaker fluorescence, while those with a higher DOS (e.g., DOS > 6) may also show reduced fluorescence. The optimal DOS for most antibodies typically ranges between 2 and 10, depending on the specific properties of both the dye and the protein. For effective labeling, it is recommended to achieve a DOS of 6-8 moles of XFD532 NHS ester per mole of antibody. The following steps outline the process for determining the DOS of XFD532 NHS ester-labeled proteins.

Measure Absorption

For accurate measurement of the absorption spectrum of a dye-protein conjugate, maintain the sample concentration between 1-10 μM, adjusting as needed based on the dye's extinction coefficient.

Read OD (absorbance) at 280 nm and Dye Maximum Absorption (λmax = 534 nm for XFD532 NHS Ester)

For most spectrophotometers, the sample (from the column fractions) needs to be diluted with de-ionized water so that the O.D. values are in the range of 0.1 to 0.9. The O.D. (absorbance) at 280 nm is the maximum absorption of protein, while 534 nm is the maximum absorption of XFD532 NHS ester. To obtain accurate DOS, ensure the conjugate is free of the non-conjugated dye.

Calculate DOS

You can calculate DOS using our tool by following this link:

https://www.aatbio.com/tools/degree-of-labeling-calculator

EXAMPLE DATA ANALYSIS AND FIGURES

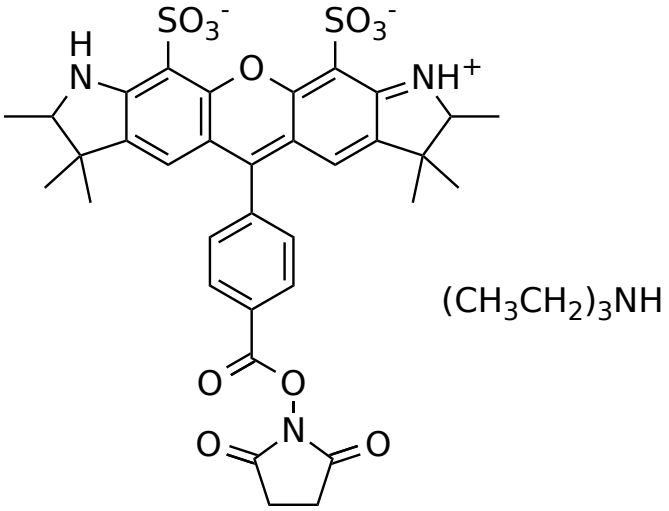

Figure 1. The chemical structure for XFD532 NHS Ester.

DISCLAIMER

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