ATTO 550 NHS ester

Catalog number: 70231
Unit size: 1 mg

Component	Storage	Amount (Cat No. 70231)
ATTO 550 NHS ester	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

ATTO 550 is an orange fluorescent dye chemically related to the well-known Rhodamine 6G and Rhodamine B dyes. It is characterized by a strong absorption, a high fluorescence quantum yield, and excellent photostability and thermal stability. This dye exhibits moderate hydrophilicity, with an optimal excitation range of 540-565 nm. ATTO 550 is cationic and carries a net electrical charge of +1 after coupling to a substrate. This dye is well-suited for advanced applications in single-molecule detection and high-resolution microscopy techniques, including PALM, dSTORM, and STED microscopy. It is also compatible with flow cytometry (FACS), fluorescence in situ hybridization (FISH), and a variety of other biological assays. ATTO 550 can be used with excitation sources and fluorescence filters similar to those for Cy3® and TAMRA.

The N-hydroxysuccinimidyl (NHS) ester of ATTO 550 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 ATTO 550 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. Mix 100 µL of a reaction buffer (e.g., 1 M sodium carbonate solution or 1 M phosphate buffer with pH ~9.0) with 900 µL of the target protein solution (e.g., antibody, protein concentration >2 mg/mL if possible) to give 1 mL protein labeling stock solution.

   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 the pH to the range of 8.0-9.0 using 1 M sodium bicarbonate solution or 1 M pH 9.0 phosphate buffer.

   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, it must be dialyzed against 1X PBS, pH 7.2-7.4, to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation.

   Note: Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well. The presence of sodium azide or thimerosal might also interfere with the conjugation reaction. Sodium azide or thimerosal can be removed by dialysis or spin column for optimal labeling results.

   Note: The conjugation efficiency is significantly reduced if the protein concentration is less than 2 mg/mL. The final protein concentration range of 2-10 mg/mL is recommended for optimal labeling efficiency.

ATTO 550 NHS ester stock solution (Solution B)

1. Add anhydrous DMSO into the vial of ATTO 550 NHS ester to make a 10 mM stock solution. Mix well by pipetting or vortex.

   Note: Prepare the dye stock solution (Solution B) before starting the conjugation. Use promptly. Extended storage of the dye stock solution may reduce the dye activity. Solution B can be stored in the freezer for two weeks when kept from light and moisture. Avoid freeze-thaw cycles.

SAMPLE EXPERIMENTAL PROTOCOL

This labeling protocol was developed for the conjugate of Goat anti-mouse IgG with ATTO 550 NHS ester. You might need further optimization for your particular proteins.

Note: Each protein requires a distinct dye/protein ratio, which also depends on the properties of dyes. Over-labeling of a protein could detrimentally affect its binding affinity, while the protein conjugates of low dye/protein ratio give reduced sensitivity.

Run conjugation reaction

1. Use a 10:1 molar ratio of Solution B (dye)/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) into the vial of 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 less 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 conjugation

The following protocol is an example of dye-protein conjugate purification by using a Sephadex G-25 column.

1. Prepare Sephadex G-25 column according to the manufacture instruction.
2. Load the reaction mixture (From "Run conjugation reaction") 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 the most important factor for characterizing dye-labeled protein. Proteins of lower DOS usually have weaker fluorescence intensity, but proteins of higher DOS (e.g., DOS > 6) tend to have reduced fluorescence too. The optimal DOS for most antibodies is recommended between 2 and 10, depending on the properties of dye and protein. For effective labeling, the degree of substitution should be controlled to have 6-8 moles of ATTO 550 NHS ester to one mole of antibody. The following steps are used to determine the DOS of ATTO 550 NHS ester-labeled proteins.

Measure Absorption

To measure the absorption spectrum of a dye-protein conjugate, it is recommended to keep the sample concentration in the range of 1-10 μM depending on the extinction coefficient of the dye.

Read OD (absorbance) at 280 nm and dye maximum absorption (λmax = 553 nm for ATTO 550 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 553 nm is the maximum absorption of ATTO 550 NHS ester. To obtain accurate DOS, ensure the conjugate is free of the non-conjugated dye.

Calculate DOS

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

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

EXAMPLE DATA ANALYSIS AND FIGURES

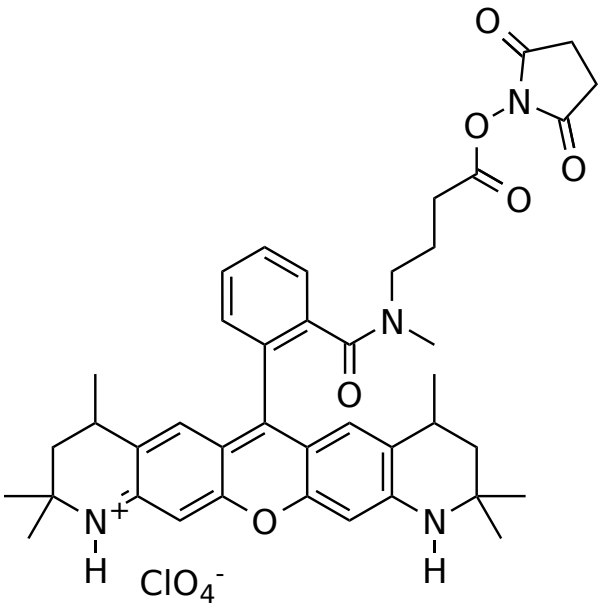

Figure 1. The chemical structure of ATTO 550 NHS ester.

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