ReadiCleave™ iFluor 594 AML-NHS ester

Catalog number: 7007
Unit size: 1 mg

Component	Storage	Amount
ReadiCleave™ iFluor 594 AML-NHS ester	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

Many biological molecules can be readily labeled with a fluorescent tag for fluorescence imaging and flow cytometry analysis. However, almost all the existing fluorescent tags are used to permanently labeling biological targets from which the added fluorescent tags cannot be cleaved for further downstream analysis, such as mass spectral analysis or another detection mode. AAT Bioquest's ReadiCleave™ linkers enable fluorescent tags conjugated to a biological target from which the added fluorescent tag can be removed when needed. ReadiCleave™ iFluor™ 594 AML uses an azidomethyl linker that can be cleaved with TCEP to remove the iFluor™ 594 fluorophore from the target molecule. The cleavage can be carried out by adding 10-100 mM TCEP solution (pH 7.5) and incubating at 65 °C for 1-5 min. iFluor™ 594 is a superior replacement to Alexa Fluor® 594. iFluor™ 594 and Alexa Fluor® 594 have very similar spectral properties.

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.

1. Protein stock solution (Solution A)
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. For optimal labeling efficiency the final protein concentration range of 2-10 mg/mL is recommended.

2. ReadiCleave™ iFluor 594 AML-NHS ester stock solution (Solution B)
Add anhydrous DMSO into the vial of ReadiCleave™ iFluor 594 AML-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 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 ReadiCleave™ iFluor 594 AML-NHS ester. You might need further optimization for your particular proteins.

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

Run conjugation reaction

1. Use 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 to use 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 need be diluted with staining buffer, and aliquoted for multiple uses.

   Note For longer term storage, dye-protein conjugate solution need be concentrated or freeze dried.

EXAMPLE DATA ANALYSIS AND FIGURES

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 ReadiCleave™ iFluor 594 AML-NHS ester to one mole of antibody. The following steps are used to determine the DOS of ReadiCleave™ iFluor 594 AML-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 = 588 nm for ReadiCleave™ iFluor 594 AML-NHS ester dyes)

For most spectrophotometers, the sample (from the column fractions) need be diluted with de-ionized water so that the OD 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 588 nm is the maximum absorption of ReadiCleave™ iFluor 594 AML-NHS ester. To obtain accurate DOS, make sure that 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

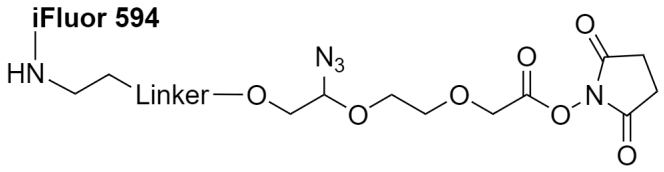

Figure 1. Chemical structure for ReadiCleave™ iFluor 594 AML-NHS ester.

DISCLAIMER

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