ReadiCleave™ AML Cy5 NHS ester

Catalog number: 7000
Unit size: 1 mg

Component	Storage	Amount
ReadiLeave™ Cy5 NHS ester	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

OVERVIEW

Fluorescence-based methods have many advantages for biological detections in terms of sensitivity. Many biological molecules are labeled with fluorescent tags for fluorescence imaging and flow cytometry analysis. However, most of the existing fluorescent tags are used to permanently labeling biological targets from which the added fluorescent tags cannot be cleaved for further downstream analysis. AAT Bioquest's ReadiCleave™ linkers enable fluorescent tags conjugated to a biological target from which the added fluorescent tag can be removed when needed. This ReadiCleave™ AML Cy5 contains an azidomethyl linker that can be cleaved with TCEP to remove the Cy5 fluorophore from the target molecule. The cleavage can be carried out by adding 10 mM TCEP solution (pH 7.5), and incubating at 65 °C for 1-5 min.

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™ AML Cy5 NHS ester stock solution (Solution B)
Add anhydrous DMSO into the vial of ReadiCleave™ AML Cy5 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™ AML Cy5 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

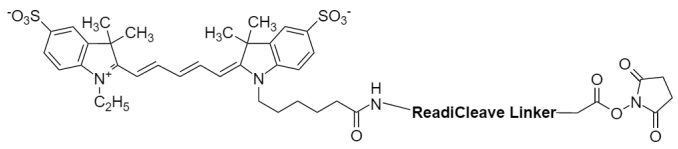

Figure 1. Chemical structure for ReadiCleave™ Cy5 NHS ester.

DISCLAIMER

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