

mFluor™ Violet 470 SE

Catalog number: 1225

Unit size: 1 mg

Component	Storage	Amount (Cat No. 1225)
mFluor™ Violet 470 SE	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

mFluor™ Violet 470 is a unique color for multicolor flow cytometry applications. Its SE is reasonably stable and shows good reactivity and selectivity with protein amino groups. mFluor™ Violet 470 SE provides a convenient tool to label monoclonal, polyclonal antibodies or other proteins (>10 kDa) for flow cytometric applications with the violet laser excitation. mFluor™ Violet 470 dyes have fluorescence excitation and emission maxima of ~405 nm and ~470 nm respectively. AAT Bioquest's mFluor™ dyes are developed for multicolor flow cytometry-focused applications. These dyes have large Stokes Shifts and can be well excited by the laser lines of flow cytometers (e.g., 405 nm, 488 nm and 633 nm).

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)

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.

mFluor™ Violet 470 SE stock solution (Solution B)

Add anhydrous DMSO into the vial of mFluor™ Violet 470 SE 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 mFluor™ Violet 470 SE. 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 affect 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.

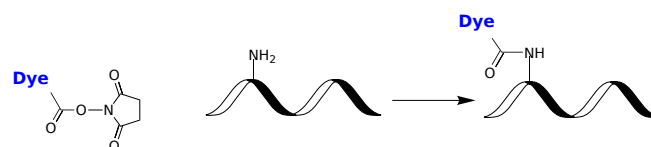
EXAMPLE DATA ANALYSIS AND FIGURES


Figure 1. Reaction scheme of Dye-NHS ester for chemical conjugation with primary amines.

DISCLAIMER

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