mFluor™ Violet 610 maleimide

Catalog number: 1613
Unit size: 1 mg

Component	Storage	Amount (Cat No. 1613)
mFluor™ Violet 610 maleimide	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

mFluor™ dyes are engineered for advanced multicolor flow cytometry applications, offering significant Stokes Shifts and optimal excitation by standard flow cytometer laser lines, such as 350 nm, 405 nm, 488 nm, 532 nm, and 633 nm. The mFluor™ Violet 610 dye, which exhibits a bright red fluorescence and moderate photostability, is ideally suited for excitation at the 405 nm laser line, with an emission maximum of ~612 nm. This maleimide derivative is commonly used for the conjugation of thiol groups on proteins, oligonucleotide thiophosphates, and low molecular weight ligands, producing conjugates that exhibit significantly brighter fluorescence and enhanced photostability compared to those formed with other spectrally similar fluorophores.

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

mFluor™ Violet 610 maleimide Stock Solution (Solution B)

1. Prepare a 10 mM mFluor™ Violet 610 maleimide stock solution by adding anhydrous DMSO to the vial of mFluor™ Violet 610 maleimide. Mix well by pipetting or vortexing.

   Note: Before starting the conjugation process, prepare the dye stock solution (Solution B) and use it promptly. Prolonged storage of Solution B may reduce its activity. If necessary, Solution B can be stored in the freezer for up to 4 weeks, provided it is protected from light and moisture. Avoid freeze/thaw cycles.

Protein Stock Solution (Solution A)

1. Prepare a 1 mL protein labeling stock solution, by mixing 100 µL of a reaction buffer (e.g., 100 mM MES buffer with a pH ~6.0) with 900 µL of the target protein solution (e.g., an antibody or protein solution with a concentration >2 mg/mL if possible).

   Note: The pH of the protein solution (Solution A) should be 6.5 ± 0.5.

   Note: Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or other proteins will not be labeled well.

   Note: The conjugation efficiency is significantly reduced if the protein concentration is less than 2 mg/mL. To achieve optimal labeling efficiency, it is recommended to maintain a final protein concentration within the range of 2-10 mg/mL.

Disulfide Reduction (If Necessary)

If your protein does not contain a free cysteine, it must be treated with DTT or TCEP to generate a thiol group. DTT and TCEP are utilized to convert disulfide bonds into two free thiol groups. If using DTT, ensure to remove any free DTT via dialysis or gel filtration before conjugating a dye maleimide to your protein. Below is a sample protocol for generating a free thiol group:

1. To prepare a fresh solution of 1 M DTT, dissolve 15.4 mg of DTT in 100 µL of distilled water.

2. To prepare the IgG solution in 20 mM DTT, first, add 20 µL of DTT stock to each milliliter of the IgG solution while mixing gently. Then, allow the solution to stand at room temperature for 30 minutes without additional mixing. This resting period helps to minimize the reoxidation of cysteines to cystines.

3. Pass the reduced IgG through a filtration column that has been pre-equilibrated with "Exchange Buffer." Collect 0.25 mL fractions as they elute from the column.

4. Determine the protein concentrations and combine the fractions containing the highest amounts of IgG. This can be accomplished using either spectrophotometric or colorimetric methods.

5. Proceed with the conjugation immediately after this step (refer to the Sample Experiment Protocol for details).

   Note: IgG solutions should be >4 mg/mL for the best results. The antibody should be concentrated if less than 2 mg/mL. Include an extra 10% for losses on the buffer exchange column.

   Note: The reduction can be carried out in almost any buffers from pH 7-7.5, e.g., MES, phosphate, or TRIS buffers.

   Note: Steps 3 and 4 can be replaced by dialysis.

SAMPLE EXPERIMENTAL PROTOCOL

This labeling protocol was designed for the conjugation of goat anti-mouse IgG with mFluor™ Violet 610 maleimide. You may need to further optimize the protocol for your specific proteins.

Note: Each protein requires a specific dye-to-protein ratio, which varies based on the properties of the dyes. Over-labeling a protein can negatively impact its binding affinity while using a low dye-to-protein ratio can result in reduced sensitivity.

Run the 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) to the vial of the protein solution (95 µL of Solution A), and mix thoroughly by shaking. The protein solution has a concentration of ~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) to Solution A (protein). If this ratio is not suitable, determine the optimal dye/protein ratio by testing 5:1, 15:1, and 20:1 ratios.

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

Purify the Conjugate

The following protocol serves as an example for purifying dye-protein conjugates using a Sephadex G-25 column.

1. Follow the manufacturer's instructions to prepare the Sephadex G-25 column.

2. Load the reaction mixture (from the "Run conjugation reaction" step) onto 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 of the resin surface.

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

   Note: For immediate use, dilute the dye-protein conjugate with staining buffer. If you need to use it multiple times, divide it into aliquots.

   Note: For long-term storage, the dye-protein conjugate solution should be either concentrated or freeze-dried.

Characterize the Desired Dye-Protein Conjugate

The Degree of Substitution (DOS) is a key factor in characterizing dye-labeled proteins. Proteins with a lower DOS generally have weaker fluorescence intensity, while those with a higher DOS may also have reduced fluorescence. For most antibodies, the optimal DOS is recommended to be between 2 and 10, depending on the properties of the dye and protein. For effective labeling, the DOS should be controlled to have 5-8 moles of mFluor™ Violet 610 maleimide per mole of antibody. The following steps outline how to determine the DOS of mFluor™ Violet 610 maleimide-labeled proteins.

Measure Absorption

To measure the absorption spectrum of a dye-protein conjugate, maintain the sample concentration between 1 and 10 µM. The exact concentration within this range will depend on the dye's extinction coefficient.

Read OD (absorbance) at 280 nm and dye maximum absorption (ƛmax = 421 nm for mFluor™ Violet 610 dyes)

For most spectrophotometers, dilute the sample (from the column fractions) with de-ionized water until the OD values fall within the range of 0.1 to 0.9. The optimal absorbance for protein is at 280 nm, while for mFluor™ Violet 610 maleimide, it is at 421 nm. To ensure accurate readings, make sure the conjugate is free of any 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

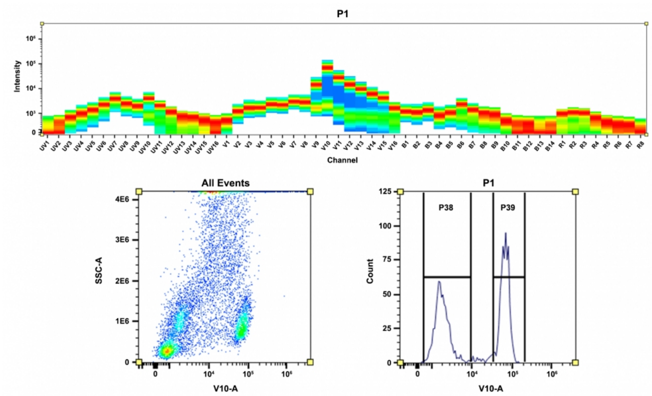

Figure 1. Top) The spectral pattern was obtained using a 4-laser spectral cytometer. Lasers at wavelengths of 355 nm, 405 nm, 488 nm, and 640 nm were spatially offset to generate four distinct emission profiles. The combination of these profiles resulted in the overall spectral signature. **Bottom)** Flow cytometry analysis was conducted on whole blood cells stained with the CD4-mFluor™ Violet 610 conjugate. The fluorescence signal was detected using an Aurora spectral flow cytometer, specifically in the V10-A channel optimized for mFluor™ Violet 610.

DISCLAIMER

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