mFluor™ Violet 500 maleimide

Catalog number: 1612
Unit size: 1 mg

Component	Storage	Amount (Cat No. 1612)
mFluor™ Violet 500 maleimide	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

mFluor™ Violet 500 Maleimide is an excellent building block that can be readily used for labeling biomolecules that have a free thiol (SH) group such as antibodies and thiol-modified oligos. mFluor™ Violet 500 dyes have fluorescence excitation and emission maxima of ~405 nm and ~500 nm respectively. These spectral characteristics make them an excellent replacement for Pacific Green™ (ThermoFisher) and BD Horizon™ V500 labeling dyes. mFluor™ Violet 500 Maleimide is reasonably stable and shows good reactivity and selectivity with free thiol group. mFluor™ Violet 500 Maleimide provides a convenient tool to label monoclonal, polyclonal antibodies or other proteins (>10 kDa) for flow cytometric applications with the violet laser excitation. Under the same conditions, mFluor™ Violet 500 dye conjugates are significantly brighter than the corresponding bioconjugates of Pacific Green™ (ThermoFisher) and BD Horizon™ V500 with much stronger absorption, making the mFluor™ Violet 500 conjugates much more sensitive. 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

mFluor™ Violet 500 maleimide Stock Solution (Solution B)

1. Prepare a 10 mM mFluor™ Violet 500 maleimide stock solution by adding anhydrous DMSO to the vial of mFluor™ Violet 500 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 500 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 500 maleimide per mole of antibody. The following steps outline how to determine the DOS of mFluor™ Violet 500 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 (λmax = 410 nm for mFluor™ Violet 500 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 500 maleimide, it is at 410 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

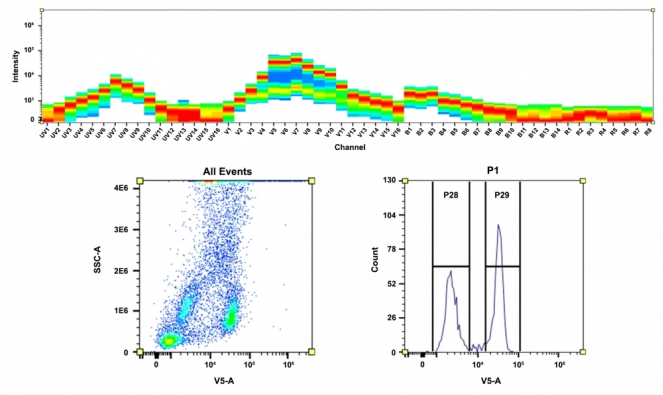

Figure 1. Top) The spectral pattern was generated using a 4-laser spectral cytometer. The lasers, spatially offset at wavelengths of 355 nm, 405 nm, 488 nm, and 640 nm, produced four distinct emission profiles. When combined, these profiles resulted in the overall spectral signature. **Bottom)** Flow cytometry analysis was performed on whole blood cells stained with CD4-mFluor™ Violet 500 conjugate. The fluorescence signal was detected using an Aurora spectral flow cytometer in the mFluor™ Violet 500-specific V5-A channel.

DISCLAIMER

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