mFluor™ Violet 530 maleimide

Catalog number: 1617
Unit size: 1 mg

Component	Storage	Amount (Cat No. 1617)
mFluor™ Violet 530 maleimide	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

mFluor™ Violet 530 dyes have fluorescence excitation and emission maxima of ~405 nm and ~530 nm respectively. These spectral characteristics make them a unique color for flow cytometry application. mFluor™ Violet 530 Maleimide is reasonably stable and shows good reactivity and selectivity with thiol group. It provides a convenient tool to label the reduced form of monoclonal, polyclonal antibodies or other proteins that contains a thiol group. These conjugates are useful for flow cytometric applications with the violet laser excitation, in particular suitable for spectral flow cytometric applications. 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., 350 nm, 405 nm, 488 nm and 633 nm). mFluor™ Violet dyes are optimized to be excited with a Violet laser at 405 nm. AAT Bioquest offers the largest collection of fluorescent dyes that are excited by Violet laser at 405 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 530 maleimide stock solution (Solution B)

Add anhydrous DMSO into the vial of mFluor™ Violet 530 maleimide 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 upto 4 weeks when kept from light and moisture. Avoid freeze-thaw cycles.

Protein stock solution (Solution A)

Mix 100 μL of a reaction buffer (e.g., 100 mM MES buffer with pH ~6.0) 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 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. For optimal labeling efficiency the final protein concentration range of 2-10 mg/mL is recommended.

Optional

If your protein does not contain a free cysteine, you must treat your protein with DTT or TCEP to generate a thiol group. DTT or TCEP are used for converting a disulfide bond to two free thiol groups. If DTT is used you must remove free DTT by dialysis or gel filtration before conjugating a dye maleimide to your protein. Following is a sample protocol for generating a free thiol group:

1. Prepare a fresh solution of 1 M DTT (15.4 mg/100 μL) in distilled water.
2. Make IgG solution in 20 mM DTT: add 20 μL of DTT stock per ml of IgG solution while mixing. Let stand at room temp for 30 minutes without additional mixing (to minimize reoxidation of cysteines to cystines).
3. Pass the reduced IgG over a filtration column pre-equilibrated with "Exchange Buffer". Collect 0.25 mL fractions off the column.
4. Determine the protein concentrations and pool the fractions with the majority of the IgG. This can be done either spectrophotometrically or colorimetrically.
5. Carry out the conjugation as soon as possible after this step (see Sample Experiment Protocol).

   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 developed for the conjugate of Goat anti-mouse IgG with mFluor™ Violet 530 maleimide. 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 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. The following steps are used to determine the DOS of mFluor™ Violet 530 maleimide 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 ($\overline{\lambda}$max = 393 nm for mFluor™ Violet 530 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 393 nm is the maximum absorption of mFluor™ Violet 530 maleimide. 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
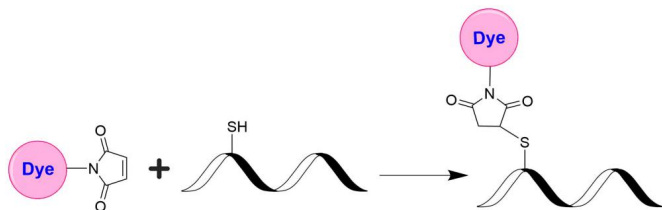

Figure 1. Fluorescent dye maleimides are the most popular tool for conjugating dyes to a peptide, protein, antibody, thiol-modified oligonucleotide, or nucleic acid through their SH group. Maleimides react readily with the thiol group of proteins, thiol-modified oligonucleotides, and other thiol-containing molecules under neutral conditions. The resulting dye conjugates are quite stable.

DISCLAIMER

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