

xtraFluor™ Violet 420 Maleimide

Catalog number: 70802, 70803
Unit size: 100 ug, 1 mg

Component	Storage	Amount (Cat No. 70802)	Amount (Cat No. 70803)
xtraFluor™ Violet 420 Maleimide	Freeze (< -15 °C), Minimize light exposure	100 ug	1 mg

OVERVIEW

xtraFluor™ Violet 420 (XV420) Maleimide is a thiol-reactive XV420 dye that can be readily used for labeling antibodies and other thiol-containing molecules such as peptides and thiol-modified oligos. XV420 is an extremely bright violet laser-excitable fluorophore. With a maximum excitation well matched the 405 nm violet laser and an emission peak at ~420 nm, XV420 can be conveniently used to replace BD Horizon Brilliant™ Violet 421 (BV421). It can be used on flow cytometers equipped with a violet laser and appropriate filters for large multicolor flow cytometry panels. It is a brighter alternative to Pacific Blue (ThermoFisher) and BD Horizon™ V450 (BD Biosciences). XV420 enables improved resolution for dim population identification. It is particularly useful in multicolor applications where it can be used to better resolve dim populations. XV420 dye offers new options for multicolor panel design through the use of a violet laser. Additionally, the dye's spillover properties, stability under light and in standard buffers, and compatibility with blood collection tubes offer ease-of-use for a range of applications.

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

xtraFluor™ Violet 420 Maleimide stock solution (Solution B)

Add anhydrous DMSO into the vial of xtraFluor™ Violet 420 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 up to 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 xtraFluor™ Violet 420 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.

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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 xtraFluor™ Violet 420 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 μ M depending on the extinction coefficient of the dye.

Read OD (absorbance) at 280 nm and dye maximum absorption (λ_{max} = 407 nm for xtraFluor™ Violet 420 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 407 nm is the maximum absorption of xtraFluor™ Violet 420 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>

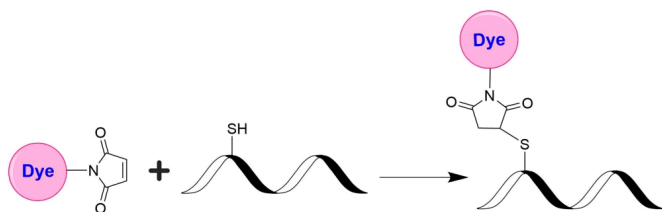


Figure 1. Fluorescent dye maleimides are the most popular tool for conjugating dyes to a peptide, protein, antibody, thiol-modified oligonucleotide or nucleic acid through their SH group. Maleimides react readily with the thiol group of proteins, thiol-modified oligonucleotides, and other thiol-containing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

DISCLAIMER

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