

xtraFluor™ Violet 420 Maleimide

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

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

AT A GLANCE

1. Add DTT to the antibody, incubate for 30 minutes at room temperature, then purify using a desalting column
2. Add xtraFluor™ Violet 420 Maleimide to the reduced antibody at a 1:1 ratio, incubate for 60-120 minutes at room temperature
3. Add NEM to block free thiols, incubate for 20 minutes, then purify the conjugate

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

Add anhydrous DMSO into the vial of xtraFluor™ Violet 420 Maleimide stock solution to make a 10 mg/mL stock solution. Mix well by pipetting or vortex.

Note: Prepare the xtraFluor™ Violet 420 Maleimide stock solution before starting the conjugation and use promptly.

SAMPLE EXPERIMENTAL PROTOCOL

Reduction of Antibody

1. Prepare a fresh solution of 1.0 M DTT (15.4 mg/100 µl) in distilled water. Antibody solutions should be at 1 mg/ml or higher for best results. The reduction can be carried out in different buffers for example: MES, phosphate, and TRIS buffers (pH range 6 to 8). The antibody should be concentrated if less than 1 mg/ml.
2. Add 2 µL of 1.0 M DTT stock per 100 µL of antibody solution, and mix well. Let the antibody solution stand at room temperature for 30 minutes without additional mixing to minimize the reoxidation of cysteines.
3. Purify the reduced antibody using a desalting column pre-equilibrated with 50 mM MES buffer (pH 6.0-6.5) and 2 mM EDTA.

(Desalting column: <https://www.aatbio.com/products/readiuse-bio-gel-p-6-spin-column?unit=60500>)

4. Measure the antibody concentration using a Nanodrop. (Con. (mg/mL) = A280 nm/1.4).

Note: Reduced antibodies are typically unstable. It is best to run the conjugation reaction immediately following purification.

Antibody Conjugation with xtraFluor™ Violet 420 Maleimide

1. Mix reduced antibody with xtraFluor™ Violet 420 Maleimide directly at the ratio of 100 µg xtraFluor™ Violet 420 Maleimide per 100µg reduced antibody. (Keep the antibody concentration lower than 2.0mg/ml, please add MES buffer to the reaction mixture if antibody concentration is higher than 2.0mg/ml).
2. Reaction for 60-120 min at room temperature.
3. After the reaction, block the free sulfhydryls on the antibody. Prepare a fresh solution of 10 mg/ml NEM in DMSO; add 3.4 µL per mg of antibody and rotate for 20 minutes at room temperature.

Conjugate Purification

1. Purify the Antibody/ xtraFluor™ Violet 420 conjugate through size exclusion chromatography (Recommended resin: Sephacryl™ High Resolution S-300 size exclusion chromatography resins).

Note: The Antibody/xtraFluor™ Violet 420 conjugate can be stored at 2-8°C and kept from light.

For long term storage, add 0.2% BSA and 0.05% Sodium Azide to the conjugate.

EXAMPLE DATA ANALYSIS AND FIGURES

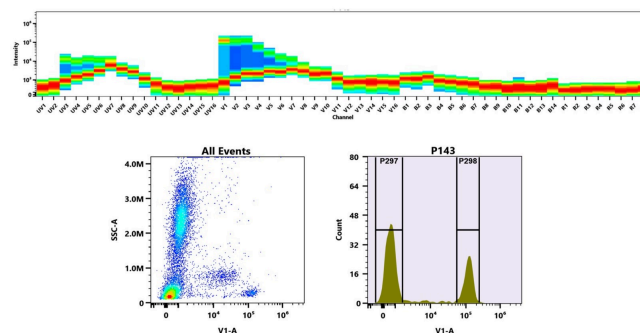


Figure 1. (Top) Spectral emission profiles generated using four spatially offset lasers (355 nm, 405 nm, 488 nm, and 640 nm). Each laser produced a distinct emission pattern, and their combination yielded the composite spectral signature. (Bottom) Flow cytometry analysis of human whole blood stained with CD4 (RPA-T4) antibody (Cat. #10041000) labeled using xtraFluor™ Violet 420 Maleimide (Cat. #70803). The fluorescence signal was monitored on a Cytek Aurora spectral flow cytometer in the V1-A channel, demonstrating clear

detection of CD4⁺ lymphocytes.

DISCLAIMER

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