xtraFluor™ Violet 420 Labeling Dye

Catalog number: 70800, 70801
Unit size: 100 ug, 1 mg

Component	Storage	Amount (Cat No. 70800)	Amount (Cat No. 70801)
Component A: xtraFluor™ Violet 420 Labeling Dye	Freeze (< -15 °C), Minimize light exposure	100 µg	1 mg
Component B: Buccutite™ MTA, NHS ester	Freeze (< -15 °C), Minimize light exposure	100 µg	100 µg
Component C: ReadiUse™ Disposable PD-10 Desalting Column		Not included	Not included

OVERVIEW

xtraFluor™ Violet 420 (XV420) is an extremely bright violet laser-excitable fluorescent labeling dye designed for flow cytometry applications requiring strong violet-channel fluorescence. With excitation and emission maxima near 405 nm and 420 nm, respectively, XV420 is well matched for instruments equipped with a 405 nm violet laser and appropriate detection filters. XV420 can be used as an alternative to BD Horizon Brilliant™ Violet 421 (BV421) and provides brighter fluorescence signal than Pacific Blue™ and BD Horizon™ V450 in violet laser-based workflows. The dye supports improved resolution of dim cell populations and is suitable for multicolor and spectral flow cytometry panel design. XV420 offers favorable spectral compatibility, fluorescence stability, and workflow compatibility for a variety of flow cytometry applications. The dye can be conjugated to antibodies using Buccutite™ chemistry developed by AAT Bioquest. Compared to conventional SMCC-based conjugation workflows, Buccutite™ chemistry enables antibody labeling under mild conditions such as room temperature and neutral pH without requiring multistep thiol reduction procedures. The resulting conjugates are suitable for flow cytometry applications requiring stable fluorescence signal and efficient violet laser excitation.

SAMPLE EXPERIMENTAL PROTOCOL

Sample Protocol for 1 mg Antibody and xtraFluor™ Violet 420 Conjugation:

Key Reagents	Details
Antibody (IgG)	1 mg in PBS, Concentration >2 mg/mL
xtraFluor™ Violet 420 Labeling Dye	1.5 mg
Buccutite™ MTA	Prepare 5 mg/mL in DMSO
Reaction Buffer (Not included)	1.0 M $NaHCO_3$ Buffer (pH=8.5)

Prepare Antibody Solution for Labeling

1. Prepare a 1 mg antibody solution (preferably at a concentration of at least 2 mg/mL) by mixing 5% (v/v) of reaction buffer (such as 1 M sodium bicarbonate solution or 1 M phosphate buffer, pH ~8.5).

   Note: The pH of the antibody solution should be around 8.0-8.5. If the pH of the antibody solution is lower than 8.0, adjust it to bring within the range using either 1 M sodium bicarbonate solution or 1 M phosphate buffer at pH 8.5.

   Note: The antibody should be dissolved in 1X phosphate-buffered saline (PBS), pH 7.2-7.4. If the protein is dissolved in Tris or glycine buffer, dialyze it against 1X PBS, pH 7.2-7.4, to remove any free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) commonly used in protein precipitation.

   Note: Antibodies that are impure or stabilized with bovine serum albumin (BSA) or gelatin may not label effectively. Additionally, sodium azide or thimerosal can interfere with the conjugation reaction. To achieve optimal labeling results, these preservatives should be removed through dialysis or spin column techniques.

   Note: For optimal labeling efficiency, it is recommended to maintain a final protein concentration between 2-10 mg/mL. Protein concentrations below 2 mg/mL can significantly reduce conjugation efficiency.

Prepare Buccutite™ MTA Stock Solution

1. Add 20 µL of anhydrous DMSO directly to the vial of Buccutite™ MTA (100 µg/vial) to prepare 5 mg/ml stock solution. Mix well by pipetting or vortexing.

   Note: Prepare the Buccutite™ MTA stock solution before starting the conjugation, and use it promptly. Extended storage of the dye stock solution may reduce the dye activity. It can be stored in the freezer for up to two weeks, provided it is protected from light and moisture. Avoid freeze-thaw cycles.

Antibody Activation with Buccutite™ MTA

1. Add Buccutite™ MTA to the antibody (refer table below) and mix well by repeatedly pipetting for a few times or vortex the vial for few seconds.

Antibody concentration	Buccutite™ MTA to be added
~ 1 mg/mL	15-20 µL
~ 2 mg/mL	10-15 µL
~ 5 mg/mL or more	5-10 µL

2. Incubate the Antibody- MTA reaction mixture at room temperature for 30 - 60 minutes.

3. Purify Antibody- MTA reaction mixture on a gel filtration column, such as a Sephadex G-25 column or equivalent matrix, or by extensive dialysis at 4°C in PBS buffer.

4. Calculate the Antibody- Buccutite™ MTA concentration by estimating 80% yield after desalting. For example, if starting with 1 mg protein, after desalting column purification, the recovery protein amount is ~ 0.8 mg.

Recommended AAT Bioquest Desalting Columns:

Volume of Reaction	Catalog #
0.6-1.0mL	Cat#60504: PD-10 Column https://www.aatbio.com/products/readiuse-disposable-pd-10-desalting-column?unit=60504
~0.1-0.15mL	Cat#60500: Spin Column https://www.aatbio.com/products/readiuse-bio-gel-p-6-spin-column?unit=60500

Combine elutions if using multiple columns.

Antibody Conjugation with xtraFluor™ Violet 420 Labeling Dye

1. Conjugation reaction is initiated by mixing Antibody- MTA and xtraFluor™ Violet 420 at the ratio of 1 mg antibody to 1.5 mg xtraFluor™ Violet 420.

   Note: Add PBS to dilute the Ab-MTA to 2 mg/ml before mixing with the dye.
2. Incubate the mixture for 1~2 hour at room temperature or overnight at 4°C. The reaction mixture is now ready to use.
3. If required, the reaction mixture can be purified by size exclusion chromatography (SEC), and the desired conjugate fractions are pooled and combined.

Characterize the SEC Purified Antibody–xtraFluor™ Violet 420 Conjugate

1. Use Nanodrop to measure the absorption spectrum of the SEC purified Antibody–xtraFluor™ Violet 420 conjugate.
2. Read OD (absorbance) at 280 nm and dye maximum absorption (λ max = 407 nm). Calculate DOL and antibody concentration with the following parameters:

   Ec (407 nm) = 3,000,000 $M^{-1}cm^{-1}$
   CF280 = 0.067

   Ec (IgG) = 210,000 $M^{-1}cm^{-1}$
3. To get the accurate antibody concentration, BCA assay is recommended (Cat. #11115).

EXAMPLE DATA ANALYSIS AND FIGURES

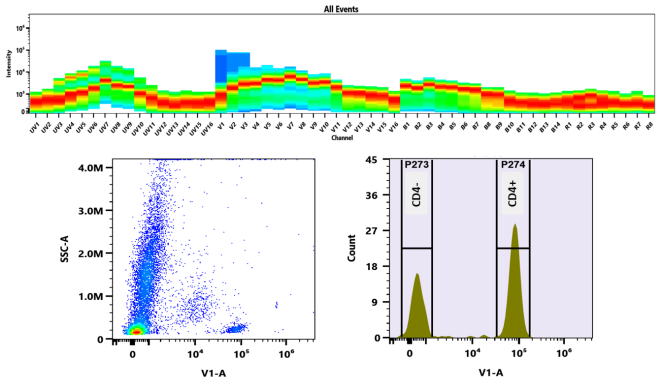

Figure 1. Top) The spectral profile was obtained using a 4-laser spectral flow cytometer equipped with spatially separated excitation sources at 355 nm, 405 nm, 488 nm, and 640 nm. Each laser generated a distinct emission pattern, which was computationally unmixed to produce the composite spectral signature of the fluorophore. Bottom) Flow cytometric analysis of human whole blood stained with xtraFluor™ Violet 420–conjugated anti-human CD4 antibody (clone RPA-T4, 0.25 µg/test). Fluorescence emission was detected in the V1-A channel using the Aurora spectral flow cytometer.

DISCLAIMER

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