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. 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. XV420 can be readily conjugated to antibodies through the well-known Buccutite™ chemistry that was developed by the scientists of AAT Bioquest. Compared to other conjugation chemistry, Buccutite™ chemistry can be run under extremely mild conditions (e.g., room temperature at neutral pH) with high conjugation yield.

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 2.5 µL-3.5 µL of 5 mg/ml Buccutite™ MTA to 1mg of antibody and mix well by repeatedly pipetting for a few times or vortex the vial for few seconds.
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

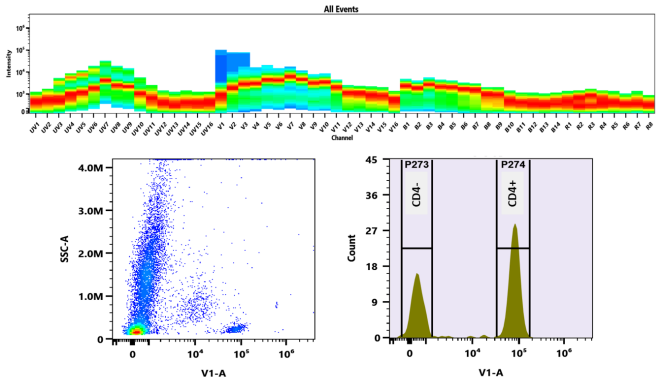

Figure 1. Top) The spectral profile was obtained using a 4-laser spectral flow cytometer equipped with spatially separated excitation sources at 355 nm, 405 nm, 488 nm, and 640 nm. Each laser generated a distinct emission pattern, which was computationally unmixed to produce the composite spectral signature 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

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.