xtraFluor™ Violet 420 Amine

Catalog number: 70806
Unit size: 200 ug

Component	Storage	Amount (Cat No. 70806)
xtraFluor™ Violet 420 Amine	Freeze (< -15 °C), Minimize light exposure	200 ug

OVERVIEW

xtraFluor™ Violet 420 Amine is a violet laser-excitable fluorescent dye with excitation and emission maxima of approximately 407 nm and 424 nm. The dye contains a primary amine functionality that supports in situ maleimide generation through reaction with heterobifunctional crosslinkers such as SMCC. This reaction produces thiol-reactive maleimide-functional XV420 intermediates that can subsequently conjugate with sulfhydryl-containing biomolecules including reduced antibodies, peptides, and proteins. XV420 provides strong fluorescence signal intensity for improved detection of dim or weakly expressed targets in complex samples. The dye is compatible with conventional and spectral flow cytometry workflows using appropriate violet laser detection configurations. Its spectral properties and high brightness support multicolor panel design requiring reduced spillover and improved population discrimination. The dye can be incorporated into custom thiol-reactive conjugation workflows requiring flexible fluorescent labeling strategies under standard bioconjugation conditions.

AT A GLANCE

1. Reduce antibody with DTT and purify by desalting
2. Activate xtraFluor™ violet 420 amine with sulfo-SMCC or SMCC
3. Purify the activated xtraFluor™ Violet 420-maleimide intermediate
4. Conjugate the activated dye to the reduced antibody
5. Purify and store the antibody conjugate protected from light

PREPARATION OF STOCK SOLUTIONS

xtraFluor™ Violet 420 Amine Stock Solution

Add 10% v/v 1.0 M sodium phosphate buffer, pH=7.5 (not provided) to the xtraFluor™ violet 420 amine vial.

SAMPLE EXPERIMENTAL PROTOCOL

Reduction of Antibody

1. Prepare a fresh 1.0 M DTT solution by dissolving 15.4 mg DTT in 100 µL distilled water.
   Note: For optimal results, the antibody concentration should be at least 1 mg/mL. Reduction can be performed in a variety of buffers, including MES, phosphate, or Tris buffers, within a pH range of 6.0–8.0. If the antibody concentration is below 1 mg/mL, concentrate the antibody prior to the reduction step.
2. Add 2 µL of freshly prepared 1 M DTT stock solution per 100 µL of antibody solution and mix thoroughly. Incubate the antibody solution at room temperature for 30 minutes without further mixing.
   Note: Following reduction, proceed promptly to the purification step to minimize reoxidation of free sulfhydryl groups.
3. Purify the reduced antibody using a desalting column pre-equilibrated with 50 mM MES buffer (pH 6.0–6.5) containing 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 spectrophotometer.
   Antibody concentration (mg/mL) = A280 / 1.4
   Note: The reduced antibody is not stable. The conjugation reaction needs to be carried out as soon as possible after purification.

Activation of xtraFluor™ Violet 420 Amine

1. Prepare a 10 mg/mL sulfo-SMCC or SMCC (not provided) stock solution in DMSO.
   Sulfo-SMCC: (https://www.aatbio.com/products/sulfo-smcc-4-n-maleimidomethyl-cyclohexane-1-carboxylic-acid-3-sulfo-n-hydroxysuccinimide-ester-sodium-salt-cas-92921-24-9?unit=4505)
   SMCC: https://www.aatbio.com/products/smcc-4-n-maleimidomethyl-cyclohexanecarboxylic-acid-n-hydroxysuccinimide-ester-cas-64987-85-5
2. Add approximately 5.0 µL of the 10 mg/mL sulfo-SMCC or SMCC stock solution per 200 µg of xtraFluor™ violet 420 amine stock solution (prepared by adding 10% v/v 1.0 M sodium phosphate buffer, pH=7.5 to the xtraFluor™ violet 420 amine vial), mix thoroughly, and incubate at room temperature for 60 minutes.
3. Purify the activated xtraFluor™ Violet 420-maleimide conjugate using a desalting column pre-equilibrated with 50 mM MES buffer (pH 6.0–6.5) containing 2 mM EDTA.
4. Measure the concentration of the Sulfo-SMCC activated xtraFluor™ violet 420 (xtraFluor™ violet 420 – maleimide) using a NanoDrop spectrophotometer.
   xtraFluor™ violet 420 concentration (mg/mL) = A407 / 30

Conjugation of Reduced Antibody with Activated xtraFluor™ Violet 420

1. Mix 100 µg of reduced antibody with 150 µg of activated xtraFluor™ Violet 420-maleimide. For optimal conjugation efficiency, keep the antibody concentration below 1.5 mg/mL. If necessary, dilute the reaction mixture with MES buffer containing 2 mM EDTA.
2. Incubate the reaction mixture at room temperature for 60–120 minutes.
3. Following conjugation, block the free sulfhydryls on the antibody by preparing a fresh 5 mg/mL NEM solution in DMSO and adding 1.0 µL per 100 µg of antibody. Mix gently and incubate at room temperature for 20 minutes.

Purification

1. The antibody/xtraFluor™ Violet 420 conjugate may be further purified by size exclusion chromatography to remove unconjugated dye and improve conjugate purity.

   Note: Store the antibody/xtraFluor™ violet 420 conjugate at 2–8 °C protected from light.

EXAMPLE DATA ANALYSIS AND FIGURES

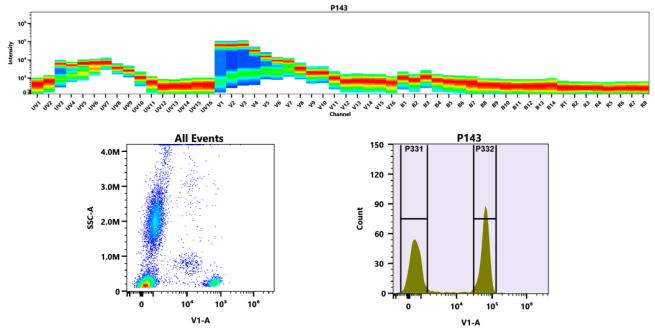

Figure 1. (Top) Spectral emission profiles generated using four spatially offset lasers (355 nm, 405 nm, 488 nm, and 640 nm). Each laser produced a distinct emission pattern, and their combination yielded the composite spectral signature. (Bottom) Flow cytometry analysis of whole blood stained with xtraFluor™ Violet 420-labeled Anti-human CD4 Antibody (Clone: RPA-T4) (Cat. #10041000) prepared using xtraFluor™ Violet 420 Amine (Cat. #70806). The fluorescence signal was monitored using Cytek Aurora spectral flow cytometer in the V1-A channel. The antibody was used at 0.5 µg/test.

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.