Buccutite™ Rapid XV420 Antibody Labeling Kit *Production Scale Optimized for Labeling 1 mg Antibody Per Reaction*

Catalog number: 70811
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 70811)
Component A: XV420 Labeling Dye	Freeze (< -15 °C), Minimize light exposure	2 vials (300 µL/vial)
Component B: Buccutite™ MTA	Freeze (< -15 °C), Minimize light exposure	2 vials (lyophilized)
Component C: Reaction Buffer	Freeze (< -15 °C)	1 vial (100 µL)
Component D: Spin Column	Room temperature (10-25 °C)	2 columns

OVERVIEW

Buccutite™ Rapid XV420 Antibody Labeling Kit is designed for conjugation of antibodies with xtraFluor™ Violet 420 (XV420), an exceptionally bright violet laser-excitable fluorophore with excitation and emission maxima of approximately 407 nm and 424 nm, respectively. XFV420 is compatible with flow cytometers equipped with a 405 nm laser and appropriate detection filters, supporting multicolor flow cytometry applications. The dye provides strong fluorescence signal and improved detection of dim populations compared to commonly used fluorophores such as Pacific Blue and BV421. The kit employs Buccutite™ conjugation chemistry to enable antibody labeling under mild conditions, including room temperature and neutral pH. The preactivated dye format allows direct conjugation without multistep crosslinking reactions commonly associated with SMCC-based methods. Each kit includes all essential components for two labeling reactions. This format supports larger-scale workflows while maintaining consistent conjugation performance for flow cytometry applications.

AT A GLANCE

Key Parameters to Achieve Best Performance

1. 1.0 mg Antibody (MW ~150 kDa)
2. Antibody concentration: 2.0 mg/mL
3. Antibody volume: 500 µL

PREPARATION OF WORKING SOLUTION

Important

Before opening the vials, warm all components and briefly centrifuge. Immediately prepare necessary solutions before starting conjugation. This protocol is a recommendation.

Prepare Antibody Solution

1. Prepare a 500 µL antibody solution in PBS with a concentration of 2 mg/mL.

   Note: The protein should be dissolved in 1X phosphate-buffered saline (PBS), pH 7.2 - 7.4. If the protein is dissolved in buffers containing primary amines, like Tris and/or glycine, it must be dialyzed against 1X PBS, pH 7.2 - 7.4, or use Amicon Ultra0.5, Ultracel-10 Membrane, 10 kDa (Cat No. UFC501008 from Millipore) to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation.

   Note: Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well.

Prepare Buccutite™ MTA Solution

1. Allow a vial of Buccutite™ MTA (Component B) to warm to room temperature.
2. Add 10 µL of DMSO (not provided) to the vial of Buccutite™ MTA (Component B), and mix well by pipetting.

SAMPLE EXPERIMENTAL PROTOCOL

Run Antibody-Buccutite™ MTA Reaction

1. Add 25 µL of Reaction Buffer (Component C) to the antibody solution.
2. Transfer 10 µL of the reconstituted Buccutite™ MTA DMSO solution into the vial of antibody solution, and mix well by pipetting.
3. Rotate the reaction mixture at room temperature for 1 hour, then purify using a desalting column.

Purify Antibody-Buccutite™ MTA Solution with Desalting Column

1. Invert the provided spin column (Component D) several times to re-suspend the settled gel and remove any bubbles.
2. Snap off the tip and place the column in a washing tube (2 mL, not provided). Remove the cap to allow the excess packing buffer to drain by gravity to the top of the gel bed.

   Note: If the column does not begin to flow, push the cap back into the column and remove it again to start the flow. Discard the drained buffer, and then place the column back into the Washing Tube.
3. Centrifuge at 1000 x g for 2 minutes in a swinging bucket centrifuge to remove the packing buffer. Then discard the buffer.
4. Apply 1-2 mL 1X PBS (pH 7.2-7.4) to the column. After each application of PBS, let the buffer drain out by gravity, or centrifuge the column for 2 minutes to remove the buffer. Discard the buffer from the collection tube. Repeat this process for 3-4 times.
5. Centrifuge at 1000 x g for 2 minutes in a swinging bucket centrifuge to remove the packing buffer. Then discard the buffer.
6. Place the column into a clean collecting tube (1.5 mL, not provided). Then, take the antibody-Buccutite™ MTA solution from step 3 of the "Run Antibody-Buccutite™ MTA Reaction" section and

load it carefully and directly into the center of the column.

7. After loading the sample, add 40 µL of 1X PBS (pH 7.2-7.4), centrifuge the column for 2 minutes at 1,000 x g, and collect the solution that contains the desired antibody-Buccutite™ MTA solution.

Run Antibody-XFV420 Conjugation Reaction

1. Warm up a vial of XFV420 Labeling Dye (Component A) to room temperature.

   Note: Each vial of XFV420 Labeling Dye contains an optimized amount of dye to label 1 mg of IgG (MW ~150 kDa) at 2 mg/mL in PBS, the kit can also be used to label other proteins (>10 kDa).

2. Add the purified Antibody-Buccutite™ MTA solution directly into the vial of XFV420 labeling dye. Rotate the mixture for 1-2 hours at room temperature.
3. The antibody-dye conjugate is now ready for immediate use or can be stored at 4°C.

Purification with Size Exclusion Chromatography Recommended

1. For optimal performance, it is recommended to purify the antibody-XV420 conjugate using size exclusion chromatography (SEC). The desired conjugate fractions are pooled together. For accurate estimation of labeled antibody concentration, BCA assay is recommended (Cat. #11115).

EXAMPLE DATA ANALYSIS AND FIGURES

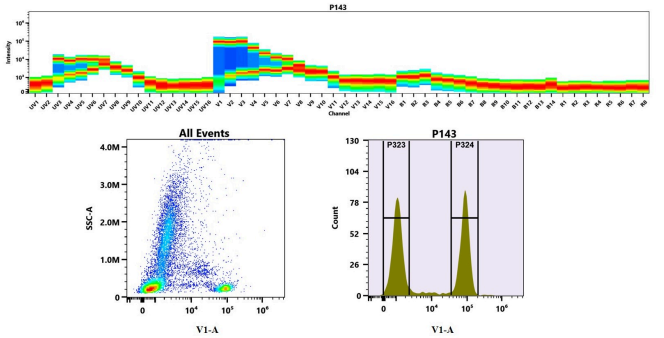

Figure 1. (Top) Spectral emission profiles generated using four spatially offset lasers (355 nm, 405 nm, 488 nm, and 640 nm). Each laser produced a distinct emission pattern, and their combination yielded the composite spectral signature. (Bottom) Flow cytometry analysis of human whole blood stained with CD4 (RPA-T4)-XV420 conjugate prepared using the Buccutite™ Rapid XV420 Antibody Labeling Kit (#70811). The fluorescence signal was monitored on a Cytek Aurora spectral flow cytometer in the V1-A channel, demonstrating clear detection of $CD4^+$ cells.

DISCLAIMER

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