Buccutite™ Rapid XV420 Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody Per Reaction*

Catalog number: 70810
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 70810)
Component A: XV420 Labeling Dye	Freeze (< -15 °C), Minimize light exposure	2 vials (10 µL/vial)
Component B: Buccutite™ MTA	Freeze (< -15 °C), Minimize light exposure	2 vials (lyophilized)
Component C: Reaction Buffer	Freeze (< -15 °C)	1 vial (20 µL)
Component D: 30K Spin Filter Set	Room temperature (10-25 °C)	2 sets

OVERVIEW

Buccutite™ Rapid XV420 Antibody Labeling Kit is designed for rapid 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. XV420 is compatible with flow cytometers equipped with a 405 nm laser and appropriate detection filters, supporting its use in multicolor flow cytometry panel design. 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 utilizes Buccutite™ conjugation chemistry, which enables antibody labeling under mild conditions, including room temperature and neutral pH. This kit is sufficient for 2 labeling reactions, each up to 25 ug of antibody. The preactivated dye format allows direct conjugation without the need for multistep crosslinking reactions typically associated with conventional chemistries such as SMCC-based methods. The resulting conjugates are suitable for flow cytometry applications requiring consistent fluorescence signal and efficient antibody labeling.

AT A GLANCE

Protocol Summary

1. Add 1.25 µL Reaction Buffer (Component C) into antibody (25 µL)
2. Add 2.5 µL Buccutite™ MTA working solution
3. Incubate at room temperature for 30 - 60 minutes
4. Use 30K Spin Filter (Component D) to purify Buccutite™ MTA activated antibody
5. Add purified Buccutite™ MTA modified antibody to XV420 (Component A)
6. Incubate at room temperature for 60-120 minutes

Important Note: Upon receipt, store Component A, B & C at -20°C. When stored properly, the kit should be stable for six months. Warm all the components and centrifuge the vials briefly before opening, and immediately prepare the required solutions before starting your conjugation. The following SOP is an example for labeling goat anti-mouse IgG antibody

PREPARATION OF WORKING SOLUTION

Antibody working solution

For labeling 25 µg antibody (assuming the target antibody concentration is 1 mg/mL), mix 1.25 µL (5% of the total reaction volume) of Reaction Buffer (Component C) with 25 µL of the target antibody solution.

Note: If you have a different concentration, adjust the antibody volume accordingly to make ~25 µg antibody available for your labeling reaction.

Note: The antibody should be dissolved in 1X phosphate buffered saline (PBS), pH 7.2-7.4; If the antibody is dissolved in glycine buffer, it must be dialyzed against 1X PBS, pH 7.2-7.4, or use Amicon Ultra-0.5, Ultracel-10 Membrane, 10 kDa (Cat. # UFC501008 from Millipore) to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for antibody precipitation.

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

Note: The antibody –Buccutite™ MTA reaction efficiency is significantly reduced if the antibody concentration is less than 1 mg/mL. For optimal labeling efficiency the final antibody concentration range of 1-10 mg/mL is recommended.

Buccutite ™ MTA working solution

Add 10 µL DMSO (Not provided) into the vial of Buccutite ™ MTA (Component B).

SAMPLE EXPERIMENTAL PROTOCOL

Run Antibody-Buccutite™ MTA reaction

1. Add 2.5 µL of Buccutite ™ MTA working solution into antibody working solution, and mix them well by repeatedly pipetting for a few times or vortex the vial for a few seconds.

2. Keep the antibody- Buccutite ™ MTA reaction mixture at room temperature for 30 - 60 minutes.

   Note: The antibody-Buccutite™ MTA reaction mixture can be incubated for longer time if desired.

Purify Buccutite™ MTA activated antibody

1. Add 400uL PBS to dilute the reaction mixture, and then transfer the reaction mixture (~425 µL) to one 30K Spin Filter (Component D), centrifuge at 6500~8000 RCF for approximately 10 minutes depending on the centrifuge device used to ~50 µL. And then add 400 µL PBS, repeat the centrifuge step two times. The Buccutite™ MTA activated antibody will be inside the membrane, and the unreacted MTA and buffer will flow through.

   Note: Save the flow-through liquid in a clean tube in case of membrane failure during centrifugation.

2. Transfer the purified Buccutite™ MTA activated antibody modified antibody carefully from inside the membrane to a fresh microcentrifuge tube, measure the volume (keep it between 20 µL-25 µL).

Make Antibody-XV420 Conjugate

1. Add Buccutite MTA activated antibody solution to one vial of XV420

Labeling Dye (Component A), mix well by repeatedly pipetting for a few times.

2. Incubate for 1-2 hours.

3. The antibody-XV420 conjugate is now ready to use.

   Note: For long term storage, add 0.1% bovine serum albumin and 0.02-0.05 % sodium azide to antibody-XV420 conjugate. The antibody-XV420 conjugate could be stored at 4 °C for six months when kept away from light.

4. Antibody concentration can be estimated with 80% yield: Ab conc. (mg/ml) = 20 µg / final volume (µL).

EXAMPLE DATA ANALYSIS AND FIGURES

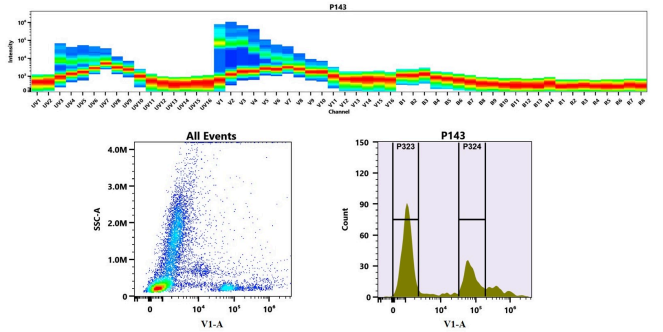

Figure 1. (Top) Spectral emission profiles generated using four spatially offset lasers (355 nm, 405 nm, 488 nm, and 640 nm). Each laser produced a distinct emission pattern, and their combination yielded the composite spectral signature. (Bottom) Flow cytometry analysis of human whole blood stained with CD4 (RPA-T4)-XV420 conjugate prepared using the Buccutite™ Rapid XV420 Antibody Labeling Kit (#70810). 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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