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

Catalog number: 5406
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 5406)
Component A: Buccutite™ FOL-Activated APC	Refrigerated (2-8 °C), Minimize light exposure	2 vials
Component B: Buccutite™ MTA	Freeze (< -15 °C), Minimize light exposure	2 vials
Component C: Reaction Buffer	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL)
Component D: Spin Column	Refrigerated (2-8 °C)	2 vials

OVERVIEW

Buccutite™ Rapid Crosslinked APC Antibody Labeling Kits, designed for large-scale production, offer a convenient and efficient method to label antibodies with APC, PE, and iFluor® tandem dyes. In comparison to traditional protein-protein conjugation methods like the SMCC crosslinking technique, Buccutite™ conjugation is simple and more robust. Using a two-step mixing protocol, researchers can directly conjugate APC to any antibody or protein in less than 2 hours. Each Buccutite™ kit includes all the essential components for two labeling reactions and features a user-friendly, pre-packed spin column to maximize conjugate yield. Each Buccutite™ FOL-Activated APC vial provided in this kit is precisely formulated to label 1 mg of purified protein or antibody. Before labeling, it's important to remove stabilizing proteins like BSA from the sample and avoid using amine-rich buffers like Tris, which might disrupt the labeling process. Allophycocyanin (APC) is an intensely bright, red fluorescent phycobiliprotein with an excitation and emission maxima of ~651 nm and ~660 nm, respectively. Given its intense brightness, APC is recommended for pairing with low-abundance targets to minimize spillover and compensation. APC conjugates are well-suited for FRET screening, flow cytometry, spectral flow cytometry, and other immunoassays requiring high sensitivity but not photostability. With Buccuitte™ Rapid Antibody Labeling kits, researchers can directly label primary antibodies, eliminating the need for secondary antibodies and enhancing panel-building flexibility.

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 5 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 5 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. Refer to the 'Centrifugation Notes' section below for instructions.
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. Refer to the 'Centrifugation Notes' section below for instructions.

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-APC Conjugation Reaction

1. Warm up a vial of Buccutite™ FOL-Activated APC (Component A) to room temperature.

   Note: Each vial of Buccutite™ FOL-Activated APC 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. Make a Buccutite™ FOL-Activated APC solution by adding 130 µL of ddH2O into the vial of Buccutite™ FOL-Activated APC (Component A), and mix well by pipetting or vortexing.

3. Add the purified Antibody-Buccutite™ MTA solution directly into the vial of Buccutite™ FOL-Activated APC solution. Rotate the mixture for 1-2 hours at room temperature.

4. The antibody-APC 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-APC conjugate using size exclusion chromatography (SEC). The following SEC columns are suitable for this purpose: Superdex 200 Increase 100/300 GL (Cytiva) and ENrich™ SEC 650 10 x 300 Column (Bio-Rad).

EXAMPLE DATA ANALYSIS AND FIGURES

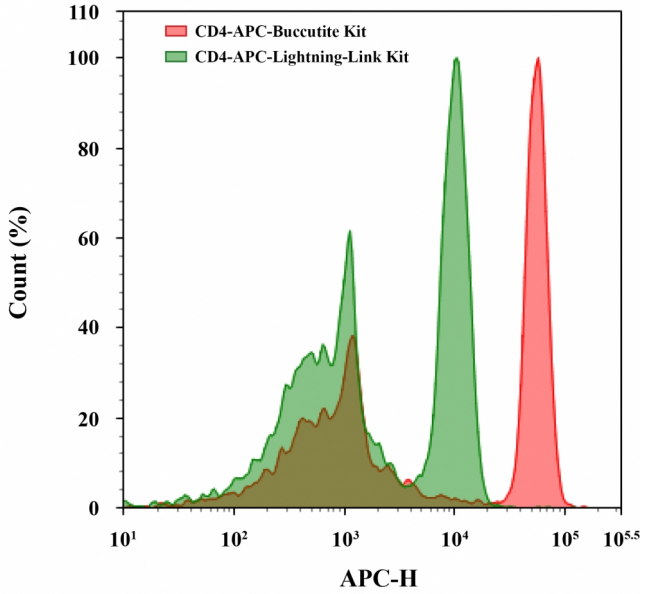

Figure 1. Flow cytometry analysis of CD4 PBMC populations. Anti-human CD4 monoclonal antibody was labeled using Buccutite™ Rapid Crosslinked APC Antibody Labeling Kit *Production Scale* (Cat No. 5406) or Lightning-Link® Rapid APC Antibody Labeling Kit according to manufacturers' instructions. CD4 PBMC populations were then stained, and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the APC channel.

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.