Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit *Production Scale Optimized for Labeling 1 mg Antibody Per Reaction*

Catalog number: 5411
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 5411)
Component A: Buccutite™ FOL-Activated PE-Cy7	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 columns

OVERVIEW

Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kits, designed for large-scale production, provide a streamlined approach for labeling antibodies with PE, APC, PerCP, and iFluor® tandem dyes. Compared to conventional 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 PE-Cy7 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 for maximum conjugate yield. Each Buccutite™ FOL-Activated PE-Cy7 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. Phycoerythrin-cyanine 7 (PE-Cy7) is an intensely bright, red fluorescent tandem fluorophore with an excitation and emission maxima of ~565 nm and ~778 nm, respectively. Given its intense brightness, PE-Cy7 is recommended for pairing with low-abundance targets to minimize spillover and compensation. PE-Cy7 conjugates are well-suited for 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. Warm up a vial of Buccutite™ MTA (Component B) 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-PE-Cy7 Conjugation Reaction

1. Warm up a vial of Buccutite™ FOL-Activated PE-Cy7 (Component A) to room temperature.

   Note: Each vial of Buccutite™ FOL-Activated PE-Cy7 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 PE-Cy7 solution by adding 250 µL of ddH2O into the vial of Buccutite™ FOL-Activated PE-Cy7 (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 PE-Cy7 solution. Rotate the mixture for 1-2 hours at room temperature.

4. The antibody-PE-Cy7 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-PE-Cy7 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

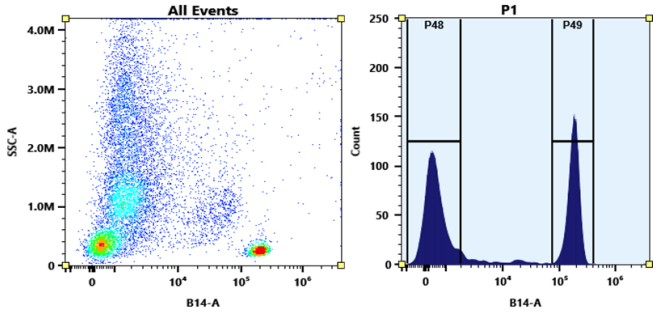

Figure 1. Flow cytometry analysis of whole blood stained with PE-Cy7 anti-human CD4 *SK3* conjugate. The fluorescence signal was monitored using an Aurora spectral flow cytometer in the PE-Cy7-specific B14-A channel. PE-Cy7 anti-human CD4 *SK3* conjugates were prepared using the Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit *Production Scale* (Cat# 5411).

DISCLAIMER

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