Buccutite™ Fc Site-Specific PerCP-Cy7 Tandem Antibody Conjugation Kit *Optimized for Labeling 1 mg Antibody*

Catalog number: 5561
Unit size: 2 labelings (1 mg each)

Component	Storage	Amount (Cat No. 5561)
Component A: Fc Glycan Modifier	Freeze (< -15 °C)	1 vial
Component B: Fc Reaction Buffer	Freeze (< -15 °C)	1 vial
Component C: Spin Desalting Column	Refrigerated (2-8 °C)	2 x 2mL
Component D: Buccutite-Fc-MTA Reagent	Freeze (< -15 °C)	2 vials
Component E: DMSO	Freeze (< -15 °C)	100 µL
Component F: Fc Reaction Deactivator	Freeze (< -15 °C)	100 µL
Component G: Buccutite™ FOL-Activated PerCP-Cy7	Refrigerated (2-8 °C), Minimize light exposure	2 lvials each)

OVERVIEW

Buccutite™ Fc Site-Specific PerCP-Cy7 Tandem Antibody Conjugation Kit is designed for selective conjugation of antibodies to PerCP-Cy7 tandem dye through Fc region-specific Buccutite™ conjugation chemistry. The Buccutite™ technology selectively targets accessible Fc glycans for controlled antibody modification while minimizing labeling within antigen-binding regions. This site-specific approach helps preserve antibody binding activity and generates more uniform antibody conjugates compared to random lysine-labeling methods. The kit is optimized for labeling 1 mg antibody per reaction and offers a cost-effective approach for preparing Fc-specific PerCP-Cy7-conjugated antibodies for downstream detection workflows. The resulting antibody-PerCP-Cy7 conjugates are suitable for flow cytometry, fluorescence-based immunoassays, and other PerCP-Cy7-based detection workflows. Buccutite™ Fc site-specific conjugation technology provides a controlled alternative to random lysine-labeling approaches for generating more uniform antibody conjugates under mild reaction conditions.

AT A GLANCE

1. Prepare 500 µL antibody in PBS with concentration 2 mg/ml.
2. Add 50 µL Fc Reaction Buffer to antibody solution (500 µL) followed by addition of 50 µL Fc Glycan Activator.
3. Incubate at room temperature for one hour.
4. Add 20 µL Fc Reaction Deactivator into above solution, and incubate 10 min at RT.
5. Add 20 µL DMSO to Buccutite™-Fc-MTA Crosslinker to dissolve the powder, add it to Fc Activated Antibody. Incubate at room temperature for 60 minutes.
6. Purify the Buccutite-Fc-MTA modified antibody with a Spin Desalting Column, collect the Fc-MTA-modified antibody.
7. Add 250 µL PBS to one vial of Buccutite™ FOL-Activated PerCP-Cy7 to reconstitute it, add Fc-MTA-modified antibody to this and mix well. Incubate at room temperature for 2 hours.
8. Purify the conjugate with SEC column, and pool the proper fractions. The conjugate is ready to use.

Important Notes:

1. Upon receipt, store Component A, B, D, E and F 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 prepare the required solutions immediately before starting your conjugation.
2. The following SOP is an example for labeling 1mg of Anti-CD4 (SK3) monoclonal antibody (mouse IgG1) (500 µL with concentration 2 mg/ml). The antibody Fc modification efficiency is best if antibody concentration is above 5 mg/ml, and will be reduced if the antibody concentration is less than 2 mg/mL.
3. The reaction volume in each step can be adjusted proportionately based on the antibody volume.

PREPARATION OF WORKING SOLUTION

Antibody Working Solution

For labeling 1 mg antibody (assuming the target antibody concentration is 2 mg/mL), prepare 500 µL antibody solution in PBS.

Notes: 1. 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 Ultracel Membrane (30 kDa from Millipore) to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for antibody precipitation.

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

3. The antibody Fc Modification efficiency is significantly reduced if the antibody concentration is less than 2 mg/mL.

Buccutite™-Fc-MTA Reagent Working Solution

Add 20 µL DMSO (Component E) into the vial of Buccutite-Fc-MTA (Component D).

Buccutite™ FOL-Activated PerCP-Cy7 Working Solution

Add 250 µL PBS into the vial of Buccutite™ FOL-Activated PerCP-Cy7 (Component G) to dissolve the powder before use.

SAMPLE EXPERIMENTAL PROTOCOL

Step 1: Fc Glycan Activation Reaction

1. Add 50 µL Fc Reaction Buffer (Component B) to antibody solution (500 µL), mix well by repeatedly pipetting for a few times.
2. Add 50 µL Fc Glycan Activator (Component A) into above antibody solution. (The total volume will be 600 µL).

   Note: If you have a different antibody concentration, please adjust Fc Glycan Activator and Fc Reaction Buffer volume proportionately.

3. Incubate the antibody Fc glycan activation reaction mixture at room temperature for one to two hours.

   Note: Incubation time longer than 2 hours is not recommended.

4. Add 20 µL Fc Reaction Deactivator (Component F) into above

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solution, and incubate for 10 min at RT.

Step 2: Modify Fc-Glycan Activated Antibody with Buccutite-Fc-MTA Crosslinker

1. Add 20 µL of Buccutite-Fc-MTA Crosslinker working solution into the Fc Glycan Activated Antibody solution, mix well by repeatedly pipetting for a few times.
2. Incubate at room temperature for 60 min.
3. Purify Buccutite-Fc-MTA modified antibody with a Spin Desalting Column (Component C). See the **protocol to purify the reaction mixture with spin desalting column** below.
4. Antibody concentration can be measured with Nanodrop:

   Antibody concentration (mg/mL) = A280/1.4

5. Measure the volume and calculate the amount of the Buccutite-Fc-MTA modified antibody collected after purification. The Buccutite-Fc-MTA modified antibody is ready to covalently link to Buccutite-FOL activated labels.

   Note: Buccutite-Fc-MTA modified antibody could be stored at 4 °C for several weeks when kept away from light.

Step 3: Fc-MTA Activated Antibody Conjugation with Buccutite™ FOL-Activated PerCP-Cy7

1. Add reconstituted Buccutite™ FOL-Activated PerCP-Cy7 (Component G) to purified Buccutite-Fc-MTA modified antibody, mix well by repeatedly pipetting for a few times.
2. Incubate for 1-2 hours at room temperature.
3. Purify the conjugate with SEC column (Example: ENrich™ SEC 650 10 x 300 Column, BioRad Cat #7801650 or similar), and pool the proper fractions.
4. Measure concentration with Nanodrop, and the conjugate is ready to use.

   Antibody-PerCP-Cy7 concentration (mg/ml) = (A280-A480*0.25)/1.4

   Note: For longer term storage, add 0.1% bovine serum albumin and 0.02-0.05% sodium azide to conjugated antibody. It can be stored at 4 °C for six months when kept away from light.

Protocol to purify the reaction mixture with Spin Desalting Column:

1. Twist off the bottom closure of the desalting column (Component C) and loosen the cap. Place the column in a 15 mL collection tube.
2. Centrifuge the column at 1,000g for 2 minutes to remove the storage solution.
3. Remove the cap and slowly add 1 mL of 1X DPBS (not provided with the kit) to the column.
4. Centrifuge at 1,000g for 2 minutes, remove the buffer. Repeat this step for 3 additional times, discarding the buffer from the collection tube.
5. Place the column on a new 15 mL collection tube, and gently apply the sample into the center of the compact resin bed.
6. Centrifuge the column at 1,000g for 2 minutes to collect the sample.

EXAMPLE DATA ANALYSIS AND FIGURES

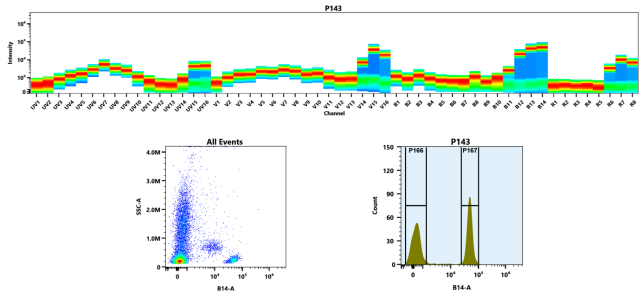


Figure 1. (Top) Spectral emission profiles generated using four spatially offset lasers (355 nm, 405 nm, 488 nm, and 640 nm). Each laser produced a distinct emission pattern, and their combination yielded the composite spectral signature of PerCP-Cy7. (Bottom) Flow cytometry analysis of whole blood stained with anti-human CD4 antibody (clone RPA-T4; Cat. #10041000) conjugated to PerCP-Cy7 using the Buccutite™ Fc Site-Specific PerCP-Cy7 Antibody Conjugation Kit (Cat. #5561). The resulting conjugate was used at 0.25 µg/test. Fluorescence was analyzed using a Cytek Aurora spectral flow cytometer in the B14-A channel.

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