Buccutite™ Fc Site-Specific APC-Cy7 Tandem Antibody Conjugation Kit *Optimized for Labeling 100 ug Protein*

Catalog number: 5586
Unit size: 2 labelings (100 ug each)

Component	Storage	Amount (Cat No. 5586)
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 1mL
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 APC-Cy7	Refrigerated (2-8 °C), Minimize light exposure	2 vials

OVERVIEW

Buccutite™ Fc Site-Specific APC-Cy7 Tandem Antibody Conjugation Kit is designed for selective conjugation of antibodies to APC-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 100 µg protein per reaction and offers a cost-effective approach for preparing Fc-specific APC-Cy7-conjugated antibodies for downstream detection workflows. The resulting antibody-APC-Cy7 conjugates are suitable for flow cytometry, fluorescence-based immunoassays, and other APC-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 50 µL antibody in PBS with concentration 2 mg/ml.
2. Add 5 µL Fc Reaction Buffer to antibody solution (50 µL) followed by addition of 5 µL Fc Glycan Activator.
3. Incubate at room temperature for one hour.
4. Add 2 µL Fc Reaction Deactivator into above solution, and incubate 10 min at RT.
5. Add 5 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 50 µL PBS to one vial of Buccutite™ FOL-Activated APC-Cy7 to reconstitute it, add Fc-MTA-modified antibody to this and mix well. Incubate at room temperature for 2 hours.
8. The Fc site-specific 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 100 µg of Anti-CD4 (SK3) monoclonal antibody (mouse IgG1). 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 100 µg antibody (assuming the target antibody concentration is 2 mg/mL), prepare 50 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 5 µL DMSO (Component E) into the vial of Buccutite-Fc-MTA (Component D).

Buccutite™ FOL-Activated APC-Cy7 Working Solution

Add 50 µL PBS into the vial of Buccutite™ FOL-Activated APC-Cy7 (Component G) to dissolve the powder before use.

SAMPLE EXPERIMENTAL PROTOCOL

Step 1: Fc Glycan Activation Reaction

1. Add 5 µL Fc Reaction Buffer (Component B) to antibody solution (50 µL), mix well by repeatedly pipetting for a few times.

2. Add 5 µL Fc Glycan Activator (Component A) into above antibody solution. (The total volume will be 60 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 2 µL Fc Reaction Deactivator (Component F) into above solution, and incubate for 10 min at RT.

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

1. Add 5 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 APC-Cy7

1. Add reconstituted Buccutite™ FOL-Activated APC-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. Estimate Antibody concentration:

   Antibody Concentration (mg/ml) = Fc- MTA Activated Antibody amount (mg) / final volume (mL)

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

DISCLAIMER

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