Buccutite™ Fc Site-Specific MTA Antibody Modification Kit *Optimized for Labeling 1mg Antibody*

Catalog number: 5572
Unit size: 2 Labelings (1 mg each)

Component	Storage	Amount (Cat No. 5572)
Component A: Fc Glycan Activator	Freeze (< -15 °C)	1 vial
Component B: Fc Reaction Buffer	Freeze (< -15 °C)	1 vial
Component C: Spin Desalting Column	Room temperature (10-25 °C)	4 x 2mL columns
Component D: Buccutite-Fc-MTA Crosslinker	Freeze (< -15 °C)	2 vials
Component E: DMSO	Freeze (< -15 °C)	100 µL

OVERVIEW

Buccutite™ Fc Site-Specific MTA Antibody Modification Kit is designed for selective MTA modification of antibodies through Fc region-specific Buccutite™ conjugation chemistry. The Buccutite™ technology selectively targets Fc glycans for controlled antibody functionalization while minimizing modification 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 provides a scalable approach for preparing Fc-MTA modified antibodies for downstream conjugation workflows. The resulting Fc-MTA modified antibodies are designed for covalent conjugation with Buccutite™ FOL-functionalized biomolecules under mild neutral reaction conditions without requiring additional catalysts. Buccutite™ MTA/FOL conjugation technology provides a controlled alternative to conventional thiol-based heterobifunctional crosslinking methods for generating stable antibody conjugates with high conjugation efficiency. The modified antibodies can be used for antibody-drug conjugates, protein-protein conjugation, imaging, and other bioconjugation applications.

AT A GLANCE

1. Add 50 µL Fc reaction buffer to antibody solution (500 µL)
2. Add 50 µL Fc glycan activator into above antibody solution, incubate at room temperature for one hour
3. Purify Fc-activated antibody with a spin desalting column
4. Add 50 µL DMSO to Buccutite-Fc-MTA crosslinker and add this reconstituted crosslinker to Fc activated antibody
5. 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. The antibody Fc site activated with MTA reaction groups is ready to covalently link to Buccutite-FOL activated labels

Important Note

Upon receipt, store Component A, B, D and E at -20^{O}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.

The following SOP is an example for labeling 1mg Anti-CD4 (SK3) monoclonal antibody (mouse IgG1).

PREPARATION OF WORKING SOLUTION

Antibody Working Solution

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

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 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.

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

Buccutite-Fc-MTA Crosslinker Working Solution

Add 10 µL DMSO (Component E) into the vial of Buccutite-Fc-MTA (Component D) to dissolve the powder.

SAMPLE EXPERIMENTAL PROTOCOL

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 (Component A) volume and Fc Reaction Buffer (Component B) volume proportionally.
3. Incubate the antibody Fc glycan activation reaction mixture at room temperature for one hour.

   Note: Longer incubation time is not recommended.
4. Purify the Fc Glycan Activated Antibody with one Spin Desalting Columns (Component C).
5. Measure the antibody concentration with nanodrop, and check the volume of antibody collected.

   Fc-Glycan-Activated Antibody amount (mg) = Con. (mg/ml) x Volume collected (mL).

Modify Fc-Glycan Activated Antibody with Buccutite-Fc-MTA Crosslinker

1. Add 10 µL of Buccutite-Fc-MTA Crosslinker working solution into the purified Fc Glycan Activated Antibody solution, mix well by repeatedly pipetting for a few times.
2. Incubate at room temperature for one hour.

Note: The reaction mixture can be incubated for longer time if desired.

3. Purify Buccutite-Fc-MTA modified antibody with one Spin Desalting Columns (Component C).

4. Antibody concentration can be estimated with 80% yield:

   Ab Con. (mg/ml) = Fc-Glycan-Activated Antibody amount (mg) x 0.8 / final volume (mL)

5. 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 if kept from light.

Purification of 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 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 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 to a new 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

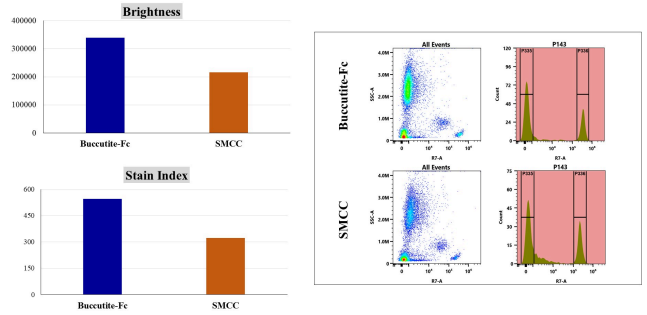

Figure 1. Comparison of conventional SMCC labeling and Fc site-specific conjugation using Anti-CD4 (SK3) APC-iFluor® 750 conjugates. Conjugates were prepared using either a regular SMCC labeling method or Buccutite™ Fc Site-Specific conjugation (#5572) and evaluated by whole blood staining. Fc site-specific conjugation demonstrated increased brightness and stain index compared to conventional SMCC labeling.

DISCLAIMER

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