

Buccutite™ Fc Site-Specific MTA Antibody Modification Kit *Optimized for Labeling 100 ug Protein*

Catalog number: 5595
Unit size: 2 labelings (100 ug each)

Component	Storage	Amount (Cat No. 5595)
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)	2 x 1 mL columns
Component D: Buccutite-Fc-MTA Crosslinker	Freeze (< -15 °C)	2 vials
Component E: DMSO	Freeze (< -15 °C)	100 µL
Component F: Fc Reaction Deactivator	Freeze (< -15 °C)	100 µL

OVERVIEW

Buccutite™ Fc Site-Specific MTA Antibody Modification Kit is designed for selective modification of antibodies with MTA groups through Fc region-specific Buccutite™ modification 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 MTA-modified antibodies for downstream conjugation workflows. The resulting MTA-modified antibodies are designed for covalent conjugation with Buccutite™ FOL-functionalized biomolecules under mild neutral conditions. Buccutite™ Fc site-specific modification 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. The Antibody Fc site activated with MTA is ready to covalently link to Buccutite-FOL activated labels.

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.

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.

Buccutite-Fc-MTA Crosslinker Working Solution

Add 5 µL DMSO (Component E) into the vial of Buccutite-Fc-MTA Crosslinker (Component D) to dissolve the powder.

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.

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 a Nanodrop:

$$\text{Antibody Concentration (mg/ml)} = \text{A280}/1.4$$

5. Measure the volume and calculate the amount of 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.

Protocol to purify the reaction mixture with Spin Desalting Column:

1. Invert the Spin Column several times to suspend the settled gel and remove any bubbles.
2. Snap off the tip and place the column in a microcentrifuge tube (2 mL). Remove the cap to allow the excess packing buffer to drain by gravity until reaching the top of the gel bed. If column does not begin to flow, push cap back into column and remove it again to start the flow. Discard the drained buffer, and then place the column back into the tube.
3. Centrifuge for 1 min in a swinging bucket centrifuge at 1,000 x g to remove the packing buffer. Discard the buffer.
4. Apply 1 mL equilibrium buffer of your choice to the column, let the buffer drain out by gravity, or centrifuge the column for 1 min to remove the buffer. Discard the buffer from the collection tube. Repeat this process for 3-4 times.
5. Centrifuge for 2 minutes in a swinging bucket centrifuge at 1,000 x g to remove the reaction buffer. Discard the buffer.
6. Place the column in a clean 2 mL microcentrifuge tube or 12 x 75 mm test tube. Carefully apply the sample (20–100 µl) directly to the top center of the column. Sample volume may need be carefully adjusted to reach the best performance.
7. After loading sample, centrifuge the column for 5 min at 1,000 x g.
8. Following centrifugation, the purified sample is now in the equilibrium buffer and molecules smaller than the column's exclusion limit (~6000) will be retained.
9. Collect the elution, and properly dispose the used column.

(SK3) either through NHS ester labeling (Lane 3, DOL ~4.0) or Fc site-specific labeling using Buccutite™ Fc Site-Specific MTA Antibody Modification Kit (#5595) (Lane 2, DOL ~2.0). Reduced conjugates were analyzed on a 4–12% denaturing gel with Coomassie Brilliant Blue staining and green fluorescence imaging

DISCLAIMER

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EXAMPLE DATA ANALYSIS AND FIGURES

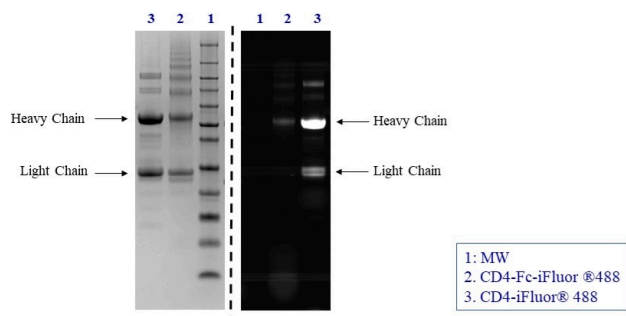


Figure 1. Comparison of conventional NHS ester labeling and Fc site-specific antibody labeling using Buccutite™ Fc Site-Specific MTA Antibody Modification Kit. iFluor® 488 was conjugated to Anti-CD4