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

Catalog number: 5571
Unit size: 2 Labelings (1 mg each)

Component	Storage	Amount (Cat No. 5571)
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-Azido Crosslinker	Freeze (< -15 °C)	2 vials
Component E: DMSO	Freeze (< -15 °C)	100 µL

OVERVIEW

Buccutite™ Fc Site-Specific Azido Antibody Modification Kit is designed for selective azide modification of antibodies through Fc region-specific Buccutite™ conjugation chemistry. The Buccutite™ technology selectively targets accessible Fc region carbohydrate residues 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 offers a cost-effective approach for preparing azide-modified antibodies for downstream click chemistry workflows. The resulting azide-modified antibodies are compatible with CuAAC and SPAAC click chemistry workflows for conjugation with AAT Bioquest alkyne-, DBCO-, and BCN-functionalized dyes, probes, and biomolecules. The modified antibodies can be used in imaging, affinity capture, and other click chemistry-based detection workflows under mild reaction conditions.

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-azido crosslinker and add this reconstituted crosslinker to Fc activated antibody
5. Incubate at room temperature for 60 minutes
6. Purify the Buccutite-Fc-azido modified antibody with a spin desalting column, collect the Fc-azido-modified antibody.
7. The Antibody Fc site activated with azido reaction groups is ready to covalently link to DIBO (dibenzocyclooctyne) activated labels

Important Note

Upon receipt, store Component A, B, D and E 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.

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-Azido Crosslinker Working Solution

Add 10 µL DMSO (Component E) into the vial of Buccutite-Fc-Azido Crosslinker (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-Azido Crosslinker

1. Add 10 µL of Buccutite-Fc- Azido 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-Azido 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- Azido modified antibody is ready to covalently link to DIBO activated labels.

   Note: Buccutite-Fc-Azido 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.