Buccutite™ Streptavidin Antibody Conjugation Kit *Optimized for Labeling 1 mg Protein*

Catalog number: 5511
Unit size: 1 Labeling

Component	Storage	Amount (Cat No. 5511)
Component A: Buccutite™ FOL-Activated Streptavidin	Freeze (< -15 °C), Minimize light exposure	1 Vial
Component B: Buccutite™ MTA	Freeze (< -15 °C), Minimize light exposure	1 Vial (lyophilized)
Component C: Reaction Buffer	Freeze (< -15 °C), Minimize light exposure	1 Vial (100 µL)
Component D: Spin Column	Refrigerated (2-8 °C)	1 Column

OVERVIEW

Buccutite™ Streptavidin Antibody Conjugation Kit is optimized for labeling 1 mg Protein. This streptavidin conjugation kit uses a simple and quick process for crosslinking streptavidin to an antibody. It can also be used to conjugate other proteins or peptides. The produced streptavidin-conjugated antibodies may be directly used in WB, ELISA, IHC without further purification. The Buccutite crosslinking technique has been proven to be one of the most effective conjugation methods for crosslinking two large molecules. The kit is one of the most effective streptavidin-antibody conjugation products. It can be used to generate conjugates of different ratios of streptavidin/antibody. The conjugate is highly stable since streptavidin and antibody is covalently connected via the highly stable amide bond.

AT A GLANCE

Key Factors for Optimal Labeling Efficiency

1. 1.0 mg Antibody (MW ~150 kDa).
2. Antibody concentration ≥ 2.0 mg/mL.

Important Note

Upon receiving the kit, it should be stored at a temperature of 4°C. When stored properly, the kit should remain stable for up to six months. If required, Components A and B can be stored at a temperature of -20°C, but it is important to avoid freezing Component C (Reaction Buffer). Before opening the vials, all components should be warmed and briefly centrifuged. Then, the required solutions should be immediately prepared before starting the conjugation process. The following is an example SOP for labeling goat anti-mouse IgG antibody.

PREPARATION OF WORKING SOLUTION

Antibody Working Solution

1. Prepare a 500 µL antibody solution in PBS with a concentration of 2 mg/mL.

   Note: The protein 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 ReadiUse™ 10KD Spin Filter (Cat. # 60502 from AAT Bioquest) 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.

   Note: Each vial of Buccutite™ FOL-Activated Streptavidin and Buccutite™ MTA is optimized to label 1 mg of IgG (MW ~150 kDa) at 2 mg/mL in PBS, but this kit can be used to label other amounts of protein.

Buccutite™ MTA Working Solution

1. Add 10 µL of DMSO (not provided) directly into the vial of Buccutite™ MTA (Component B).

SAMPLE EXPERIMENTAL PROTOCOL

Run Antibody-Buccutite™ MTA Reaction

1. Add 25 µL of the Reaction Buffer (Component C) to the antibody working solution.
2. Transfer 5 µL of the reconstituted Buccutite™ MTA DMSO solution into the vial of antibody working solution, and mix well by pipetting several times or vortexing the vial for a few seconds.
3. Rotate the antibody-Buccutite™ MTA reaction mixture for 1 hour at room temperature.

   Note: The antibody-Buccutite™ MTA reaction mixture can be rotated or shaken for a longer time if desired.

Purify Antibody-Buccutite™ MTA Solution with Desalting Column

1. Invert the provided spin column (Component D) several times to re-suspend the settled gel and remove any bubbles.
2. Snap off the tip and place the column in a washing tube (2 mL, not provided). Remove the cap to allow the excess packing buffer to drain by gravity to the top of the gel bed.

   Note: If the column does not begin to flow, push the cap back into the column and remove it again to start the flow. Discard the drained buffer, and then place the column back into the Washing Tube.
3. Centrifuge at 1000 x g for 2 minutes in a swinging bucket centrifuge to remove the packing buffer. Then discard the buffer. Refer to the 'Centrifugation Notes' section below for instructions.
4. Apply 1-2 mL 1X PBS (pH 7.2-7.4) to the column. After each application of PBS, let the buffer drain out by gravity, or centrifuge the column for 2 minutes to remove the buffer. Discard the buffer from the collection tube. Repeat this process for 3-4 times.
5. Centrifuge at 1000 x g for 2 minutes in a swinging bucket centrifuge to remove the packing buffer. Then discard the buffer.

Refer to the 'Centrifugation Notes' section below for instructions.

6. Place the column into a clean collecting tube (1.5 mL, not provided). Then, take the antibody-Buccutite™ MTA solution from step 3 of the "Run Antibody-Buccutite™ MTA Reaction" section and load it carefully and directly into the center of the column.

7. After loading the sample, add 40 µL of 1X PBS (pH 7.2-7.4), centrifuge the column for 2 minutes at 1,000 x g, and collect the solution that contains the desired antibody-Buccutite™ MTA solution.

Run Antibody-Streptavidin Conjugation

1. Make a Buccutite™ FOL-Activated Streptavidin solution by adding 200 µL ddH_2O into the vial of Buccutite™ FOL-Activated Streptavidin (Component A), mix well by repeatedly pipetting for a few times or vortexing the vial for a few seconds.

2. Add the purified antibody-Buccutite™ MTA solution to the bottle of reconstituted Buccutite™ FOL-Activated Streptavidin solution, mix well and rotate the mixture for 2 hours at room temperature.

3. The antibody-streptavidin conjugate is now ready to use.

   Note: The antibody concentration is ~1.42mg/ml.

Puri cation with Size Exclusion Chromatography Recommended

1. Purifying the antibody-streptavidin conjugate using size exclusion chromatography (SEC) is recommended for optimal performance. The following SEC columns are suitable for this purpose: Superdex 200 Increase 100/300 GL (Cytiva) and ENrich™ SEC 650 10 x 300 Column (Bio-Rad).

Storage of Antibody-Streptavidin Conjugate

The antibody-streptavidin conjugate should be stored at > 0.5 mg/mL in the presence of a carrier protein (e.g., 0.1% bovine serum albumin). For longer storage, the antibody-streptavidin conjugates could be lyophilized and stored at ≤ –20 °C.

DISCLAIMER

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