Buccutite™ Streptavidin Antibody Conjugation Kit *Optimized for Labeling 100 ug Protein*

Catalog number: 5510
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 5510)
Component A: Buccutite™ FOL-Activated Streptavidin	Freeze (< -15 °C), Minimize light exposure	2 Vials (lyophilized)
Component B: Buccutite™ MTA	Freeze (< -15 °C), Minimize light exposure	2 Vials (lyophilized)
Component C: Reaction Buffer	Freeze (< -15 °C), Minimize light exposure	1 Vial (20 µL)

OVERVIEW

Buccutite™ Streptavidin Antibody Conjugation Kit is optimized for labeling 100 ug 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

Protocol Summary

1. Add 5 µL Reaction Buffer (Component C) into antibody (100 µL)
2. Add the antibody solution into Buccutite™ MTA vial (Component B)
3. Incubate at room temperature for 30 minutes
4. Mix with 50 µL Buccutite™ FOL-Activated Streptavidin (Component A)
5. Incubate at room temperature for 60 minutes

Important Note

Upon receiving the kit, it should be stored at a temperature of 4°C. When stored properly, the kit will remain stable for a period of six months. Alternatively, it is possible to store components A and B at a temperature of -20°C. Before opening the vials, it is recommended to warm all the components and briefly centrifuge them. Afterward, proceed to immediately prepare the required solutions before starting your conjugation. The following SOP serves as an example for labeling goat anti-mouse IgG antibody.

PREPARATION OF WORKING SOLUTION

Antibody Working Solution

1. To label 100 µg of antibody (assuming the target antibody concentration is 1 mg/mL), mix 5 µL (5% of the total reaction volume) of the Reaction Buffer (Component C) with 100 µL of the target antibody solution.

   Note: If you have a different concentration, adjust the antibody volume accordingly to make ~100 µg antibody available for your labeling reaction.

   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 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: The antibody –Buccutite™ MTA reaction efficiency is significantly reduced if the antibody concentration is less than 1 mg/mL. For optimal labeling efficiency, the final antibody concentration range of 1-10 mg/mL is recommended.

Buccutite ™ MTA Working Solution

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

SAMPLE EXPERIMENTAL PROTOCOL

Run Antibody-Buccutite™ MTA Reaction

1. Add the antibody working solution directly into the vial of Buccutite™ MTA (Component B), and mix them well by repeatedly pipetting a few times or vortexing the vial for a few seconds.
2. Keep the antibody-Buccutite™ MTA reaction mixture at room temperature for 30 - 60 minutes.

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

Run Antibody-Streptavidin Conjugation

1. Add the antibody-Buccutite™ MTA reaction mixture directly into the vial of Buccutite™ FOL-Activated Streptavidin (Component A). The total volume should be 110 µL. After adding, mix well by repeatedly pipetting a few times or vortexing the vial for a few seconds.
2. Incubate for 1-2 hours.
3. The antibody-streptavidin conjugate is now ready to use.

   Note: The antibody concentration is 0.91 mg/mL.

Storage of Antibody-Streptavidin Conjugate

The antibody conjugate should be stored at > 0.5 mg/mL in the presence of a carrier protein (e.g., 0.1% bovine serum albumin). The Streptavidin-Antibody conjugate solution could be stored at 4 °C for two months without significant change and kept from light. For longer storage, the Streptavidin-antibody conjugates could be lyophilized and

stored at ≤ –20 °C.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.