

Buccutite™ Streptavidin Antibody Conjugation Kit *Optimized for Labeling 25 ug Protein*

Catalog number: 5509
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 5509)
Component A: Buccutite™ FOL-Activated Streptavidin	Freeze (< -15 °C), Minimize light exposure	2 Vials
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 25 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 1.25 µL Reaction Buffer (Component C) into antibody (25 µL).
2. Add 2.5 µL reconstituted Buccutite™ MTA (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 4°C. Proper storage is important to ensure that the kit remains stable for up to six months. Alternatively, Components A and B can be stored at -20°C. However, please do not freeze the Reaction Buffer (Component C). Before opening the vials, it is recommended to warm all the components and briefly centrifuge them. Then, immediately prepare the required solutions before starting the conjugation process. The following SOP provides an example of how to label goat anti-mouse IgG antibodies.

PREPARATION OF WORKING SOLUTION

Antibody Working Solution

1. To label 25 µg of antibody (assuming the target antibody concentration is 1 mg/mL), mix 1.25 µL (5% of the total reaction volume) of Reaction Buffer (Component C) with 25 µL of the target antibody solution.

Note: If you have a different concentration, adjust the antibody volume accordingly to make ~25 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.

Buccutite™ MTA Working Solution

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

SAMPLE EXPERIMENTAL PROTOCOL

Run Antibody-Buccutite™ MTA Reaction

1. Add 2.5 µL of the Buccutite™ MTA working solution to the antibody working solution. Mix thoroughly by pipetting or vortexing.
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 to the vial of the Buccutite™ FOL-Activated Streptavidin (Component A). The total volume should be 27.5 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 at room temperature.
3. The Streptavidin-antibody conjugate is now ready to use.

Note: The antibody concentration is 0.91 mg/mL.

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

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.