Buccutite™ Peroxidase (HRP) Antibody Conjugation Kit *Optimized for Labeling 100 ug Protein*

Catalog number: 5503
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 5503)
Component A: Buccutite™ FOL-Activated HRP	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

Protein-protein conjugations are commonly performed with a bifunctional linker (such as the commonly used SMCC), having different reactivity on each end for linking two different proteins. One end of the crosslinker reacts (via NHS ester) with amines (-NH2) found in the amino acid lysine and N-terminus, and the other end reacts (via maleimide) with the thiol groups (-SH) found in the amino acid cysteine. However, SMCC-modified protein is extremely unstable and often self-reactive since proteins often contain both amine and thiol groups that cause significant amount of homo-crosslinking. In addition it is quite difficult and tedious to quantify the number of maleimide groups on a protein. Buccutite™ Peroxidase (HRP) Antibody Conjugation Kit is designed for preparing horseradish peroxidase (HRP) conjugates directly from proteins, peptides, and other ligands that contain a free amino group. The HRP provided in our kit has been pre-activated with our proprietary linker Buccutite™ FOL, and can be directly used for conjugation. The Buccutite™ FOL-activated HRP readily reacts with Buccutite™ MTA-containing molecules under extremely mild neutral conditions without any catalyst required. Compared to commonly used SMCC and other similar technologies, our Buccutite™ bioconjugation system is much more robust and easier to use. It enables faster and quantitative conjugation of biomolecules with higher efficiencies and yields.

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 HRP (Component A)
5. Incubate at room temperature for 60 minutes

Important Note

Before opening the vials, it is recommended to warm all the components to room temperature and briefly centrifuge them. 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.

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 for a few times or vortex 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.

Make HRP-Antibody Conjugation

1. Make HRP- Buccutite™ FOL solution by adding 50 µL ddH_2O into the vial of Buccutite™ FOL-Activated HRP (Component A), mix well by repeatedly pipetting for a few times or vortex the vial for a few seconds.
2. Mix whole vial of Buccutite™ FOL-Activated HRP solution into the antibody- Buccutite™ MTA solution, mix well and rotating the mixture for 1 hour at room temperature.
3. The HRP-antibody conjugate is now ready to use.

   Note: For immediate use, dilute the HRP-antibody conjugate with a buffer of your choice.

   Note: Alternatively, add antibody-Buccutite™ MTA solution mixture to the vial of Buccutite™ FOL-Activated HRP directly.

Storage of HRP-Antibody 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 HRP-Antibody conjugate solution could be stored at 4 °C for two months and kept away from light. For longer storage, the HRPantibody conjugates could be lyophilized and stored at ≤ –20 °C.

EXAMPLE DATA ANALYSIS AND FIGURES

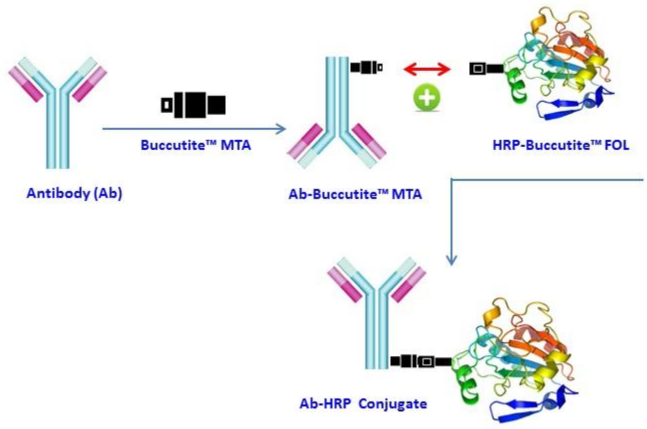

Figure 1. The mechanism of Buccutite™ bioconjugation system used for Buccutite™ Peroxidase Antibody Conjugation Kit (Cat# 5503).

DISCLAIMER

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