

## Peroxidase (HRP) Antibody Conjugation Kit

**Cat#: orb867209 (Protocol)**

### Component Storage Amount

Component A: Buccutite™ FOL-Activated HRP Refrigerated (2-8 °C), Minimize light exposure 1 vial (lyophilized) 5 vials (lyophilized)

Component B: Buccutite™ MTA Refrigerated (2-8 °C), Minimize light exposure 1 vial (lyophilized) 5 vials (lyophilized)

Component C: Reaction Buffer Refrigerated (2-8 °C), Minimize light exposure 1 vial (200 µL) 1 vial (1 mL)

### 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 cross linker reacts (via NHS ester) with amines (-NH<sub>2</sub>) 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 Buccutit™ FOL, and can be directly used for conjugation. The entire process only requires two simple mixings without further purification required. 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 10 µL Reaction Buffer (Component C) into antibody solution (200 µL) if antibody concentration is 5 mg/mL in PBS
2. Add 10 µL DMSO to Buccutite™ MTA vial (Component B) to make Buccutite™ MTA working solution
3. Add 4.5 µL Buccutite™ MTA stock solution into the antibody solution
4. Incubate at room temperature for 30 minutes
5. Add 500 µL H<sub>2</sub>O to reconstitute Buccutite™ FOL-Activated HRP (Component A)
6. Mix the Buccutite™ MTA activated antibody solution with 500 µL Buccutite™ FOL-Activated HRP (Component A)

7. Incubate at room temperature for 60 minutes

**Important** Upon receipt, store the kit at 4 °C. When stored properly, the kit should be stable for six months. Alternatively Components A and B can be stored at -20 °C. Do not freeze Reaction Buffer (Component C). Warm all the components and centrifuge the vials briefly before opening, and immediately prepare the required solutions before starting your conjugation. The following SOP is an example for labeling goat anti-mouse IgG antibody.

### PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

#### Buccutite™ MTA stock solution

Add 10 µL of DMSO into the vial of Buccutite™ MTA (Component B).

**Note** This stock solution should be used promptly.

### PREPARATION OF WORKING SOLUTION

#### Antibody working solution

For labeling 1 mg antibody (assuming the target antibody concentration is 5 mg/mL), mix 10 µL of Reaction Buffer (Component C) with 200 µL of the target antibody solution. If your antibody is not 5 mg/ml, please add 5% of total volume of Reaction Buffer (Component C).

**Note** The protocol assuming the target antibody concentration is 5 mg/mL. The antibody –Buccutite™ MTA reaction efficiency is significantly reduced if the antibody concentration is less than 1 mg/mL. For optimal labeling efficiency the antibody concentration range of 1-5 mg/mL is recommended.

**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 10KD Spin Filter 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.

## SAMPLE EXPERIMENTAL PROTOCOL

### Run Antibody-Buccutite™ MTA reaction

1. Add 4.5 µL Buccutite™ MTA stock solution to antibody working solution, 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 longer time if desired.

### Make HRP-antibody conjugation

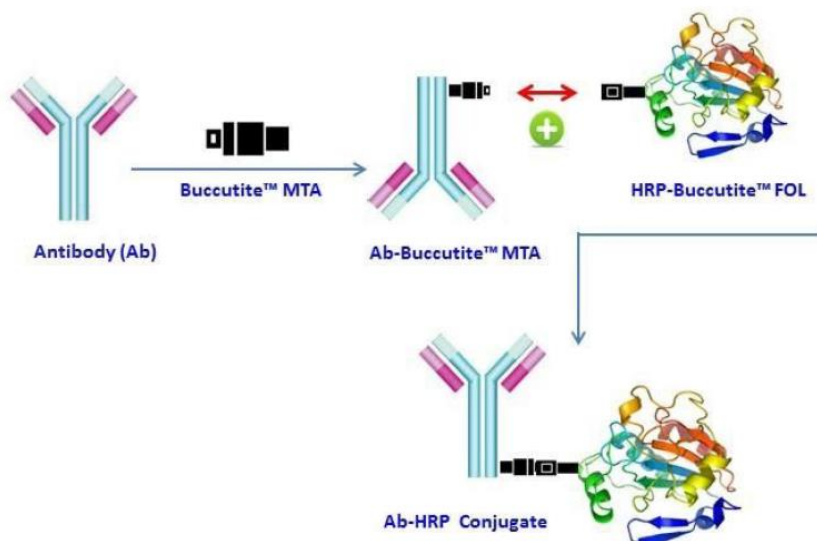
1. Make HRP- Buccutite™ FOL solution by adding 500 µL ddH<sub>2</sub>O into the vial of Buccutite™ FOL-Activated HRP (Component A), mix well by repeatedly pipetting for a few times or vortex the vial for a few seconds.
2. Mix whole vial of Buccutite™ FOL-Activated HRP solution into the antibody- Buccutite™ MTA solution, mix well and rotating the mixture for 1 hour at room temperature.
3. The HRP-antibody conjugate is now ready to use. Optional: HRP-antibody conjugate can be further purified through size exclusion chromatography to get better performance.

**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 from light. For longer storage, the HRP-antibody conjugates could be lyophilized and stored at ≤ -20 °C.

## EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.**

The mechanism of Buccutite™ bioconjugation system used for Peroxidase Antibody Conjugation Kit.