

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

Catalog number: 5503

Unit size: 1 kit

Component	Storage	Amount
Component A: Buccutite™ FOL-Activated HRP	Refrigerate (2-4 °C), Minimize light exposure	1 vial (lyophilized)
Component B: Buccutite™ MTA	Refrigerate (2-4 °C), Minimize light exposure	1 vial (lyophilized)
Component C: Reaction Buffer	Refrigerate (2-4 °C), Minimize light exposure	1 vial (20 µL)
Component D: Spin Column	Room temperature	1 column

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
4. Remove free Buccutite™ MTA by spin column
5. Mix with 50 µL Buccutite™ FOL-Activated HRP (Component A)
6. Incubate at room temperature

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) and Spin Column (Component D). 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 WORKING SOLUTION

Antibody working solution:

For labeling 100 µg antibody (assuming the target antibody concentration is 1 mg/mL), mix 5 µL (5% of the total reaction volume) of 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 Amicon Ultra-0.5, Ultracel-10 Membrane, 10 kDa (Cat. # UFC501008 from Millipore) 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 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 longer time if desired.

Prepare spin column for antibody-Buccutite™ MTA purification

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. If column does not begin to flow, push cap back into column and remove it again to start the flow. Discard the drained buffer, and then place the column back into the Washing Tube. However, centrifuge immediately if the column is placed into a 12 x 75 mm test tube (not provided).
3. Centrifuge for 2 minutes in a swinging bucket centrifuge at 1,000 x g (see Centrifugation Notes section) to remove the packing buffer. Discard the buffer.
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 for 2 minutes in a swinging bucket centrifuge at 1,000 x g (see Centrifugation Notes section) to remove the packing buffer. Discard the buffer.

Purify the antibody-Buccutite™ MTA solution

1. Place the column (from Step Prepare spin column for antibody-Buccutite™ MTA purification) in a clean Collecting Tube (1.5 mL, not provided). Carefully load the sample (~105 µL, from Step Antibody-Buccutite™ MTA reaction) directly to the center of the column.
2. After loading the sample, add 5 µL of 1X PBS (pH 7.2-7.4) to make the total volume of 110 µL. Centrifuge the column for 5-6 minutes at 1,000 x g, and collect the solution that contains the desired antibody-Buccutite™ MTA solution.

Make HRP-antibody conjugation

1. Make HRP- Buccutite™ FOL solution by adding 50 µL ddH₂O into the vial of HRP- Buccutite™ FOL (Component A), mix well by repeatedly pipetting for a few times or vortex the vial for a few seconds.
2. Mix whole vial of HRP- Buccutite™ FOL solution (from Step 5.1) into the purified antibody- Buccutite™ MTA solution (from Step Purify 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, the HRP-antibody conjugate need be diluted with the buffer of your choice.

Note For longer term storage, HRP-antibody conjugate solution need be concentrated or freeze dried.

Storage of HRP-Antibody Conjugate

The antibody conjugate should be stored at > 0.5 mg/mL in the presence of a carrier antibody (e.g., 0.1% bovine serum albumin). The HRP-Antibody conjugate solution could be stored at 4 °C for two months without significant change when stored in the presence of 2 mM sodium azide and kept from light. For longer storage, the HRP-antibody conjugates could be lyophilized and stored at ≤ -20 °C.

Centrifugation Notes

Spin column (Component D) can fit into 2 mL microcentrifuge tubes or 12 x 75 mm test tubes for sample collection during centrifugation. Use the 2 mL microtube with the columns for the initial column equilibration step.

Swinging bucket centrifuges capable of generating a minimum force of 1,000 x g are suitable for Bio-Spin column use. The gravitational force created at a particular revolution speed is a function of the radius of the microcentrifuge rotor. Consult the swinging bucket centrifuge instruction manual for the information about conversion from revolutions per minute (RPM) to centrifugal or g-force. Alternatively, use the following equation to calculate the speed in RPM required to reach the gravitational force of 1,000 x g.

$RCF (x g) = (1.12 \times 10^{-5}) \times (RPM)^2 \times r$ (RCF is the relative centrifugal force, r is the radius in centimeters measured from the center of the rotor to the middle of the Bio-Spin column, and RPM is the speed of the rotor).

EXAMPLE DATA ANALYSIS AND FIGURES

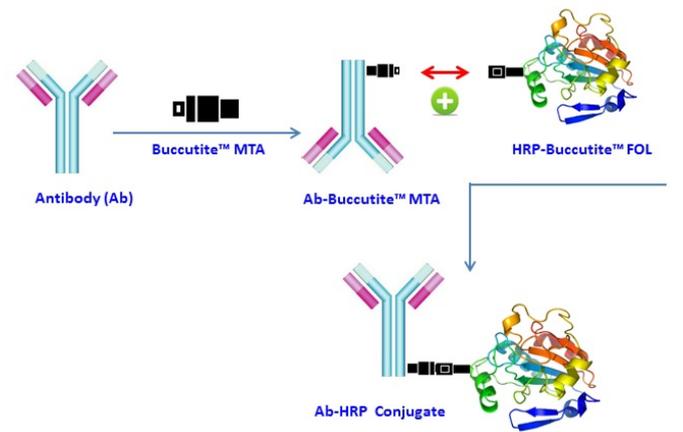


Figure 1.

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

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.