Buccutite™ Rapid PE Antibody Labeling Kit

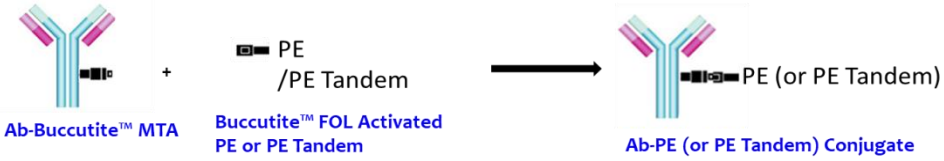
Microscale Optimized for Labeling 100 ug Antibody Per Reaction

Ordering Information	*Storage Conditions*
Cat#: 1310, 1316, 1317, 1318, 1322 (2 conjugations)	Refrigerator (2-8 °C)

Introduction

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 (-NH_2) 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™ PE Antibody Conjugation Kit is designed for preparing PE conjugates directly from proteins, peptides, and other ligands that contain a free amino group. The PE 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 PE 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.



Kit Components

Components	Amount	Storage
Component A: Buccutite™ FOL-Activated PE or PE Tandem	2 Vials (lyophilized)	4 °C
Component B: Buccutite™ MTA	2 Vials (lyophilized)	4 °C
Component C: Reaction Buffer	1 Vial (20 µL)	4 °C (Do not freeze)
Component D: Spin Column	2 Columns	4 °C (Do not freeze)

Standard Operating Protocol (Labeling 100 µg Antibody)

Upon receipt, store the kit at 4 °C. When stored properly, the kit should be stable for six months. Alternatively Components B can be stored at -20°C. Do not freeze Buccutite™ FOL-Activated PE or PE Tandem (Component A), 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.

Brief Summary

Add 5 µL reaction buffer (Component C) into antibody (100 µL) → Add the antibody solution into Buccutite™ MTA vial (Component B) → Incubate at room temperature → Remove free Buccutite™ MTA by spin column → Mix with 50 µL Buccutite™ FOL-Activated PE or PE Tandem (Component A) → incubate at room temperature

1. Prepare antibody 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 1: *If you have a different antibody concentration, adjust the antibody volume accordingly to make ~100 µg antibody available for your labeling reaction.*

Note 2*: 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 protein precipitation.*

Note 3*: Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well.*

Note 4*: The Antibody –Buccutite™ MTA reaction efficiency is significantly reduced if the antibody concentration is lessthan 1 mg/mL. For optimal labeling efficiency the final antibody concentration range of 1-10 mg/mL is recommended.*

2. Run Antibody-Buccutite™ MTA reaction:

2.1 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.

2.2 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.*

3. Prepare spin column for Antibody-Buccutite™ MTA purification:

3.1 Invert the provided spin column (Component D) several times to re-suspend the settled gel and remove any bubbles.

3.2 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).

3.3 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.

3.4 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.

3.5 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.

4. Purify the Ab-Buccutite™ MTA solution:

4.1 Place the column (from Step 3.5) in a clean Collecting Tube (1.5 mL, not provided). Carefully load the sample (~105 µL, from Step 2.2) directly to the center of the column.

4.2 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 protein-Buccutite™ MTA solution.

5. Make Ab-PE or PE Tandem conjugation:

5.1 Mix whole vial of Buccutite™ FOL-Activated PE or PE Tandem (Component A) with the purified Ab- Buccutite™ MTA solution (from Step 4.2), and rotate the mixture for 1 hour at room temperature.

5.2 The Ab-PE or PE Tandem conjugate is now ready to use.

Note 1: *For immediate use, the Ab-PE or PE Tandem conjugate need be diluted with the buffer of your choice.*

Note 2: *The concentration of the conjugate is about 0.5~0.6 mg Ab/mL if start with 100uL 1mg/ml antibody solution.*

Storage of Ab-PE or PE Tandem 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) and 0.02-0.05% sodium azide. The Ab-PE or PE Tandem conjugate solution could be stored at 4 °C for two months without significant change and kept from light.

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 micro-centrifuge 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) = $(1.12 \times 10^{-5}) \times (\text{RPM}) \times 2 \times r$ (*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*).

Table 1. Buccutite™ Rapid PE Antibody Labeling Kit (2 Conjugations/Kit, Each Labeling is for 100 µg Antibody)

Cat. #	Product Name	PE Tandems
1310	Buccutite™ Rapid PE Antibody Labeling Kit	PE
1316	Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit	PE-Cy5.5
1317	Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit	PE-Cy7
1318	Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kit	PE-Texas Red
1322	Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kit	PE-Cy5

References

1. Duncan, R.J.S., *et al.* (1983). A new reagent which may be used to introduce sulfhydryl groups into proteins, and its use in the preparation of conjugates for immunoassay. *Anal Biochem* **132:**68-73.
2. Yoshitake, S., *et al.* (1979). Conjugation of glucose oxidase from *Aspergillus niger* and rabbit antibodies using *N*-hydroxysuccinimide ester of *N*-(4-carboxycyclohexyl-methyl)maleimide. *Eur J Biochem* **101:**395-9.
3. Hashida, S., *et al.* (1984). More useful maleimide compounds for the conjugation of Fab' to horseradish peroxidase through thiol groups in the hinge. *J Appl Biochem* **6:**56-63.
4. Imagawa, M., *et al.* (1982). Characteristics and evaluation of antibody- horseradish peroxidase conjugates prepared by using a maleimide compound, glutaraldehyde, and periodate. *J Appl Biochem* **4:**41-57.