Buccutite™ Rapid PE Antibody Labeling Kit *Microscale Optimized for Labeling 100 ug Antibody Per Reaction*

Catalog number: 1310
Unit size: 2 Labelings

Component	Storage	Amount
Component A: Buccutite™ FOL-Activated PE	Refrigerated (2-8 °C), Minimize light exposure	2 vials (lyophilized)
Component B: Buccutite™ MTA	Refrigerated (2-8 °C), Minimize light exposure	2 vials (lyophilized)
Component C: Reaction Buffer	Refrigerated (2-8 °C), Minimize light exposure	1 Vial (20 µL)

OVERVIEW

R-Phycoerythrin (PE) is an orange fluorescent protein which has an excitation wavelength of 565 nm and an emission wavelength of 575 nm. AAT Bioquest offers this Buccutite™ rapid labeling kit to facilitate the PE conjugations to antibodies and other proteins such as streptavidin and other secondary reagents. Buccutite™ PE Conjugation Kit provides a robust and convenient method to conjugate your antibodies with PE. The kit includes a preactivated PE and reaction buffer. The conjugated antibody can be used in WB, ELISA and IHC applications. This kit is sufficient for 2 labeling reactions, each up to 100 ug of antibody. Considering the large size of PE (240 kDa), the amount of antibody used in a labeling reaction must always be less than the amount of PE. The best ratio for any new antibody reagent must be determined by experimentation but 50-60 ug of IgG antibody for every 100 ug of PE usually gives optimal results. Our kit provides preactivated PE to facilitate the PE conjugations to antibodies and other proteins such as streptavidin and other secondary reagents. Our preactivated PE is ready to conjugate, giving much higher yield than the conventionally tedious SMCC-based conjugation chemistry. In addition, our preactivated PE is conjugated to a protein via its amino group that is abundant in proteins while SMCC chemistry targets the thiol group that has to be regenerated by the reduction of antibodies.

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 PE (Component A)
5. 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, Component B can be stored at -20°C. Do not freeze Buccutite™ FOL-Activated PE (Component A), 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 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 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 longer time if desired.

Make antibody-PE conjugation

1. Make Buccutite™ FOL-Activated PE solution by adding 50 µL ddH2O into the vial of Buccutite™ FOL-Activated PE (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 PE solution into the antibody-Buccutite™ MTA solution, mix well and rotating the mixture for 1 hour at room temperature.

3. The antibody-PE conjugate is now ready to use.

   Note For immediate use, the antibody-PE conjugate need be diluted with the buffer of your choice.

Storage of Antibody-PE 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 Antibody-PE 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 antibody-PE conjugates could be lyophilized and stored at ≤ –20 °C.

Table 1. Available fluorophores at AAT Bioquest Buccutite™ Rapid Antibody Labelling Kits

Cat#	Labels	Ex (nm)	Em (nm)
1310	PE	565	575
1322	PE-Cy5	565	674
1316	PE-Cy5.5	565	700
1317	PE-Cy7	565	780
1318	PE-Texas Red	565	600
1311	APC	651	662
1319	APC-iFluor™ 700	651	713
1320	APC-Cy5.5	651	700
1321	APC-Cy7	651	780
1325	PerCP	482	677

EXAMPLE DATA ANALYSIS AND FIGURES

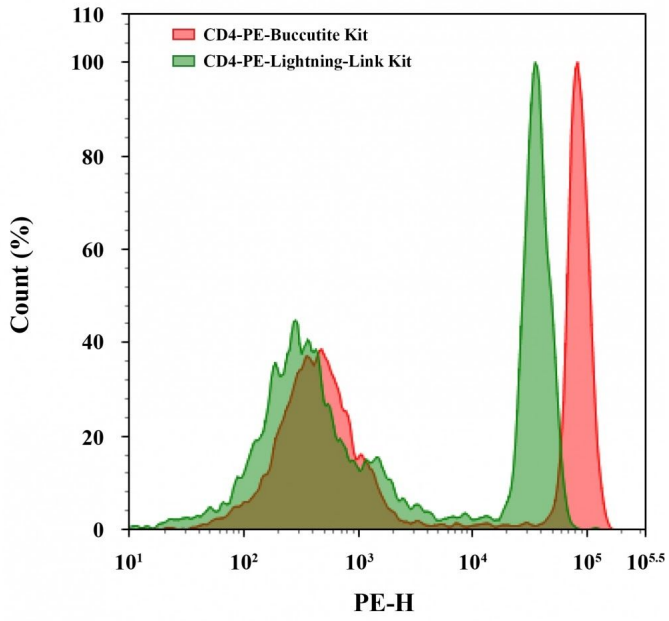

Figure 1. Flow cytometry analysis of CD4 PBMC populations. Anti-human CD4 monoclonal antibody was labeled using Buccutite™ Rapid PE Antibody Labeling Kit (Cat No. 1310) or Lightning-Link® Rapid PE Antibody Labeling Kit according to manufacturers' instructions. CD4 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the PE channel.

DISCLAIMER

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