Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 ug Antibody Per Reaction*

Catalog number: 1322
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 1322)
Component A: Buccutite™ FOL-Activated PE-Cy5	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

PE-Cy5 is a popular color used in flow cytometry. Its primary absorption peak is at 565 nm with emission peak at 674 nm. The filter sets of 682/33 nm and 695/40 nm are recommended for this tandem color. AAT Bioquest offers this Buccutite™ rapid labeling kit to facilitate the PE-Cy5 tandem conjugations to antibodies and other proteins such as streptavidin and other secondary reagents. Buccutite™ PE-Cy5 Conjugation Kit provides a robust and convenient method to conjugate your antibodies with PE. The kit includes an activated PE and reaction buffer. The conjugated antibody can be used in flow cytometry, 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 RPE. 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 RPE usually gives optimal results. Our kit provides preactivated PE-Cy5 to facilitate the PE-Cy5 tandem conjugations to antibodies and other proteins such as streptavidin and other secondary reagents. Our preactivated PE-Cy5 tandem is ready to conjugate, giving much higher yield than the conventionally tedious SMCC-based conjugation chemistry. In addition, our preactivated PE-Cy5 tandem 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-Cy5 (Component A)
5. Incubate at room temperature for 60 minutes

Important Note

Upon receipt, store the kit at 4 ^{o}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-Cy5 (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-Cy5 conjugation

1. Make Buccutite™ FOL-Activated PE-Cy5 solution by adding 50 µL ddH2O into the vial of Buccutite™ FOL-Activated PE-Cy5 (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-Cy5 solution into the antibody-Buccutite™ MTA solution, mix well and rotating the mixture for 1 hour at room temperature.

3. The antibody-PE-Cy5 conjugate is now ready to use.

   Note: For immediate use, the antibody-PE-Cy5 conjugate need be diluted with the buffer of your choice.

Storage of Antibody-PE-Cy5 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-Cy5 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-Cy5 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

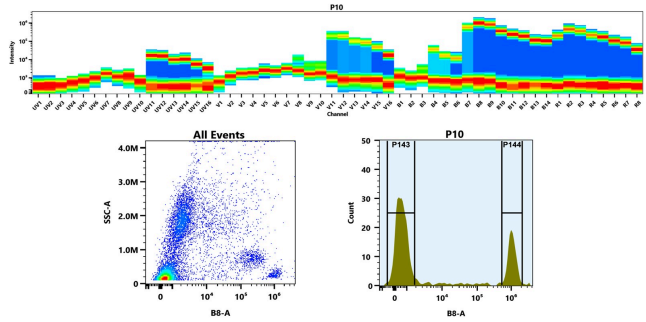

Figure 1. (Top) Spectral emission profiles generated using four spatially offset lasers (355 nm, 405 nm, 488 nm, and 640 nm). Each laser produced a distinct emission pattern, and their combination yielded the composite spectral signature. (Bottom) Flow cytometry analysis of human whole blood stained with Anti-human CD4 Antibody (SK3) labeled using Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kit (Cat. #1322). The fluorescence signal was monitored on a Cytek Aurora spectral flow cytometer in the B8-A channel, demonstrating clear detection of $CD4^+$ cells.

DISCLAIMER

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