Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody Per Reaction*

Catalog number: 1341
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 1341)
Component A: Buccutite™ FOL-Activated PE-Cy5.5	Refrigerated (2-8 °C), Minimize light exposure	2 vials
Component B: Buccutite™ MTA	Refrigerated (2-8 °C), Minimize light exposure	2 vials
Component C: Reaction Buffer	Refrigerated (2-8 °C), Minimize light exposure	1 vial (20 µL)

OVERVIEW

PE-Cy5.5 is a popular color used in flow cytometry. Its primary absorption peak is at 565 nm with emission peak at~700 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-Cy5.5 tandem conjugations to antibodies and other proteins such as streptavidin and other secondary reagents. Buccutite™ PE-Cy5.5 Conjugation Kit provides a robust and convenient method to conjugate your antibodies with PE. The kit includes a preactivated PE and reaction buffer. The entire process only requires two simple mixings without further purification required. 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 25 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 25 ug of IgG antibody for every 50 ug of PE usually gives optimal results. Our kit provides preactivated PE-Cy5.5 to facilitate the PE-Cy5.5 tandem conjugations to antibodies and other proteins such as streptavidin and other secondary reagents. Our preactivated PE-Cy5.5 tandem is ready to conjugate, giving much higher yield than the conventionally tedious SMCC-based conjugation chemistry. In addition, our preactivated PE-Cy5.5 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 1.25 µL Reaction Buffer (Component C) into antibody (25 µL)
2. Add 2.5 µL Buccutite™ MTA working solution
3. Incubate at room temperature for 30 - 60 minutes
4. Mix with 50 µL Buccutite™ FOL-Activated PE-Cy5.5 working solution
5. Incubate at room temperature for 60 minutes

Important Note

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 WORKING SOLUTION

Antibody working solution

For labeling 25 µg antibody (assuming the target antibody concentration is 1 mg/mL), mix 1.25 µL (5% of the total reaction volume) of Reaction Buffer (Component C) with 25 µL of the target antibody solution.

Note: If you have a different concentration, adjust the antibody volume accordingly to make ~25 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.

Buccutite ™ MTA working solution

Add 10 µL DMSO (Not provided) into the vial of Buccutite ™ MTA (Component B).

Note: Buccutite™ MTA working solution is unstable after reconstitution. Please use promptly upon preparation and discard the remaining portion after use.

Buccutite ™ FOL-Activated PE-Cy5.5 working solution

Add 50 µL ddH_2O into the vial of Buccutite ™ FOL-Activated PE-Cy5.5 (Component A).

SAMPLE EXPERIMENTAL PROTOCOL

Run Antibody-Buccutite™ MTA reaction

1. Add 2.5 µL of Buccutite ™ MTA working solution into 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 Antibody-PE-Cy5.5 conjugation

1. Add 50 µL of Buccutite™ FOL-Activated PE-Cy5.5 working solution with Antibody-Buccutite™ MTA solution, mix well by repeatedly pipetting for a few times or vortex the vial for a few seconds.
2. Incubate for 1 to 2 hours.
3. The antibody-PE-Cy5.5 conjugate is now ready to use.

   Note: For immediate use, the antibody-PE-Cy5.5 conjugate need

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be diluted with the buffer of your choice.

Note: For longer term storage, antibody-PE-Cy5.5 conjugate solution need be concentrated or freeze dried.

Storage of Antibody-PE-Cy5.5 Conjugate

The antibody conjugate should be stored in the presence of a carrier protein (e.g., 0.1% bovine serum albumin) and 0.02-0.05% sodium azide. The Ab-PE-Cy5.5 conjugate solution could be stored at 4 °C for two months without significant change and kept from light.

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

Cat#	Labels	Ex (nm)	Em (nm)
1312	PE	565	575
1340	PE-Cy5	565	674
1341	PE-Cy5.5	565	700
1342	PE-Cy7	565	780
1343	PE-Texas Red	565	600
1313	APC	651	662
1347	APC-iFluor® 700	651	713
1350	APC-Cy5.5	651	700
1351	APC-Cy7	651	780
1353	PerCP	482	677
1348	APC-iFluor® 750	651	791
1355	PE-iFluor® 594	565	606
1357	PE-iFluor® 710	565	747

EXAMPLE DATA ANALYSIS AND FIGURES

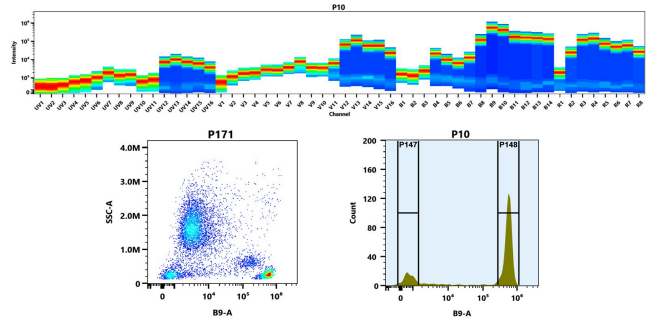


Figure 1. (Top) Spectral emission profiles generated using four spatially offset lasers (355 nm, 405 nm, 488 nm, and 640 nm). Each laser produced a distinct emission pattern, and their combination yielded the composite spectral signature. (Bottom) Flow cytometry analysis of human whole blood stained with Anti-human CD4 Antibody (SK3) labeled using Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit (Cat. #1341). The fluorescence signal was monitored on a Cytek Aurora spectral flow cytometer in the B9-A channel, demonstrating clear detection of $CD4^+$ cells.

DISCLAIMER

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