Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 ug Antibody Per Reaction*

Catalog number: 1316
Unit size: 2 Labelings

Component	Storage	Amount
Component A: Buccutite™ FOL-Activated PE-Cy5.5	Refrigerate (2-4 °C), Minimize light exposure	2 vials (lyophilized)
Component B: Buccutite™ MTA	Refrigerate (2-4 °C), Minimize light exposure	2 vials ((lyophilized)
Component C: Reaction Buffer	Refrigerate (2-4 °C), Minimize light exposure	1 vial (20 µL)
Component D: Spin Column	Room temperature	2 columns

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 an activated 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 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-Cy5.5 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 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 PE-Cy5.5 (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, Component B can be stored at -20°C. Do not freeze Buccutite™ FOL-Activated PE-Cy5.5 (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.

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.

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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 antibody-PE-Cy5.5 conjugation

1. Make Buccutite™ FOL-Activated PE-Cy5.5 solution by adding 50 µL ddH_2O into the vial of Buccutite™ FOL-Activated PE-Cy5.5 (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-Cy5.5 solution 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 antibody-PE-Cy5.5 conjugate is now ready to use.

   Note For immediate use, the antibody-PE-Cy5.5 conjugate need 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 at > 0.5 mg/mL in the presence of a carrier antibody (e.g., 0.1% bovine serum albumin). The Antibody-PE-Cy5.5 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-Cy5.5 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) = $(1.12 \times 10^{-5}) \times (RPM) \times 2 \times r$ (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. Avialble 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

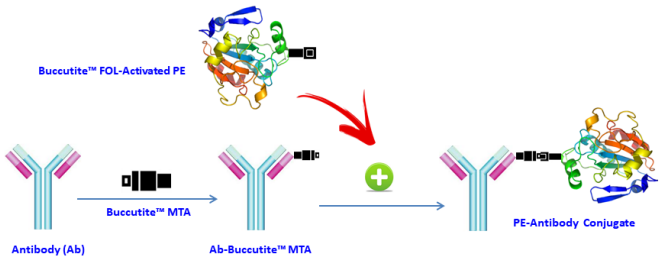

Figure 1.

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. Our preactivated PE was premodified with our Buccutite™ FOL. Your antibody (or other proteins) is modified with our Buccutite™ MTA to give MTA-modified protein (such as antibody). The MTA-modified protein readily reacts with FOL-modified PE to give the desired PE-antibody conjugate in much higher yield than the SMCC chemistry. In addition, our preactivated PE reacts with MTA-modified biopolymers at much lower concentrations than the SMCC chemistry.

DISCLAIMER