Rapid APC Antibody Labeling Kit

Cat#: orb867211 (Protocol)

Component Storage Amount
Component A: Buccutite™ FOL-Activated APC 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

APC is an red fluorescent protein which has an excitation wavelength of 651 nm and an emission wavelength of 662 nm. Biorbyt offers this Buccutite™ rapid labeling kit to facilitate the APC conjugations to antibodies and other proteins such as streptavidin and other secondary reagents. Buccutite™ APC Conjugation Kit provides a robust and convenient method to conjugate antibodies with APC. The kit includes a preactivated APC 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. The best ratio for any new antibody reagent must be determined by experimentation. Our kit provides preactivated APC to facilitate the APC conjugations to antibodies and other proteins such as streptavidin and other secondary reagents. Our preactivated APC is ready to conjugate, giving much higher yield than the conventionally tedious SMCC-based conjugation chemistry. In addition, our preactivated APC 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 APC (Component A)
5. Incubate at room temperature for 60 minutes

Important 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 APC (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 10KD Spin Filter 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-APC conjugation

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

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

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

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

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

Labels	Ex (nm)	Em (nm)
PE	565	575
PE-Cy5	565	674
PE-Cy5.5	565	700
PE-Cy7	565	780
PE-Texas Red	565	600
APC	651	662
APC-iFluor™ 700	651	713
APC-Cy5.5	651	700
APC-Cy7	651	780
PerCP	482	677

EXAMPLE DATA ANALYSIS AND FIGURES

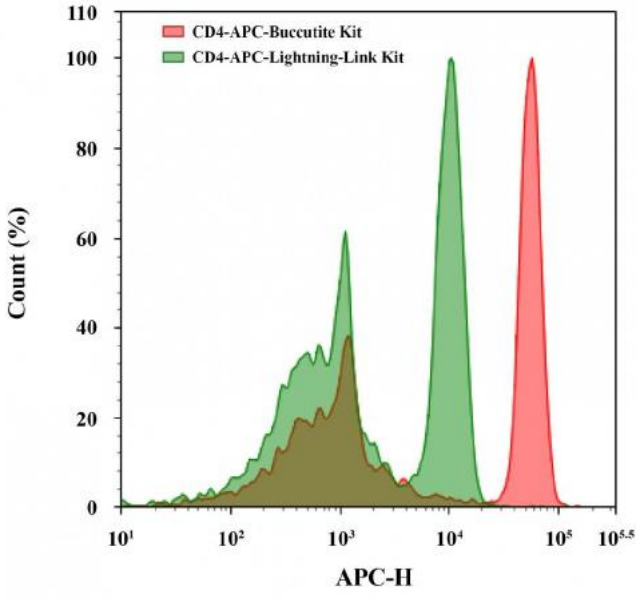

Figure 1. Flow cytometry analysis of CD4 PBMC populations. Anti-human CD4 monoclonal antibody was labeled using Buccutite™ Rapid APC Antibody Labeling Kit or Lightning-Link® Rapid APC Antibody Labeling Kit according to manufacturers' instructions. CD4 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the APC channel.