

Buccutite™ Rapid APC-iFluor® 750 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody Per Reaction*

Catalog number: 1348
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 1348)
Component A: Buccutite™ FOL-Activated APC-iFluor® 750	Freeze (< -15 °C), Minimize light exposure	2 vials
Component B: Buccutite™ MTA	Refrigerated (2-8 °C), Minimize light exposure	1 vial
Component C: Reaction Buffer	Refrigerated (2-8 °C), Minimize light exposure	1 vial (20 µL)

OVERVIEW

APC-iFluor® 750 Tandem is an excellent replacement for APC-Cy7 and APC-Alexa Fluor® 750 Tandem since they have almost identical spectra. On some antibodies, our APC-iFluor® 750 Tandem is much brighter than APC-Cy7, APC-H7, and APC-Alexa Fluor® 750 Tandem with a higher stain index. AAT Bioquest offers this Buccutite™ rapid labeling kit to facilitate the APC-iFluor® 750 tandem conjugations to antibodies and other proteins such as streptavidin and other secondary reagents. Buccutite™ APC-iFluor® 750 Tandem Conjugation Kit provides a robust and convenient method to conjugate antibodies with APC-iFluor® 750 tandem. The kit includes a preactivated APC-iFluor® 750 tandem and reaction buffer. The entire process only requires two simple mixings without further purification required. The conjugated antibody can be used in WB, ELISA, and IHC applications. This kit is sufficient for 2 labeling reactions, each up to 25 µg of antibody. The best ratio for any new antibody reagent must be determined by experimentation. Our kit provides preactivated APC-iFluor® 750 tandem to facilitate the APC-iFluor® 750 tandem conjugations to antibodies and other proteins such as streptavidin and other secondary reagents. Our preactivated APC-iFluor® 750 tandem is ready to conjugate, giving a much higher yield than the conventionally tedious SMCC-based conjugation chemistry. In addition, our preactivated APC-iFluor® 750 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 APC-iFluor® 750 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).

Buccutite™ FOL-Activated APC-iFluor™ 750 working solution

Add 50 µL ddH₂O into the vial of Buccutite™ FOL-Activated APC-iFluor® 750 (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-APC-iFluor™ 750 conjugation

1. Add 50 µL of Buccutite™ FOL-Activated APC-iFluor® 750 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-APC-iFluor® 750 conjugate is now ready to use.

Note: For immediate use, the antibody-APC-iFluor® 750 conjugate need be diluted with the buffer of your choice.

Note: For longer term storage, antibody-APC-iFluor® 750 conjugate solution need be concentrated or freeze dried.

Storage of Antibody-APC-iFluor® 750 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-APC-iFluor® 750 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

EXAMPLE DATA ANALYSIS AND FIGURES

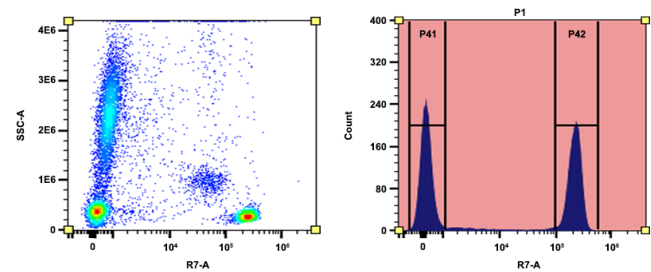


Figure 1. Flow cytometry analysis of whole blood cells stained with APC/iFluor® 750 anti-human CD4 antibody (Clone: SK3). The fluorescence signal was monitored using an Aurora flow cytometer in the APC/iFluor® 750 specific R7-A channel.

DISCLAIMER

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