Buccutite™ Rapid PE-iFluor® 710 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody Per Reaction*

Catalog number: 1357
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 1357)
Component A: Buccutite™ FOL-Activated PE-iFluor® 710	Refrigerated (2-8 °C), Minimize light exposure	2 Vials (Lyophilized)
Component B: Buccutite™ MTA	Freeze (< -15 °C), Minimize light exposure	1 Vial (Lyophilized)
Component C: Reaction Buffer	Freeze (< -15 °C), Minimize light exposure	1 Vial (20 uL)

OVERVIEW

The Buccutite™ Rapid PE-iFluor® 710 Tandem Antibody Labeling Kit offers a highly efficient and reproducible method for small-scale conjugation of antibodies with PE-iFluor® 710. Utilizing the advanced Buccutite™ crosslinking platform, this kit significantly simplifies the labeling workflow compared to conventional strategies such as SMCC-mediated crosslinking. The streamlined two-step protocol allows for the rapid conjugation of antibodies or proteins to PE-iFluor® 710 in under two hours, with minimal hands-on time.

Each kit includes all reagents necessary for two labeling reactions, with each reaction optimized to conjugate 25 µg of purified antibody or protein using Buccutite™ FOL-Activated PE-iFluor® 710. For optimal performance, the removal of stabilizing proteins (e.g., BSA) and avoidance of amine-containing buffers such as Tris are critical, as these components can interfere with the conjugation chemistry.

PE-iFluor® 710 is a tandem fluorophore with excitation and emission maxima at ~565 nm and ~747 nm, respectively, offering a substantial Stokes shift and high fluorescence intensity. These properties make it particularly well-suited for applications such as multicolor flow cytometry, spectral flow cytometry, and other fluorescence-based immunoassays requiring high sensitivity. However, due to its limited photostability, it is not recommended for applications involving prolonged light exposure. This kit enables direct labeling of primary antibodies, eliminating the need for secondary antibody-based detection systems. The resulting conjugates reduce experimental complexity and enhance assay sensitivity and reproducibility.

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-iFluor® 710 working solution
5. Incubate at room temperature for 60 minutes

Important: 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

1. To label 25 µg of 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 your antibody has a different concentration, adjust the volume to ensure approximately 25 µg of antibody is available for the 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: Ensure the antibody is pure and not stabilized with bovine serum albumin (BSA) or gelatin, as these stabilizers significantly impair labeling efficiency.

   Note: For optimal labeling efficiency, the antibody concentration should be in the range of 1–10 mg/mL. If the concentration is below 1 mg/mL, the Buccutite™ MTA reaction efficiency is significantly reduced.

Buccutite™ MTA Working Solution

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

Buccutite™ FOL-Activated PE-iFluor® 710 Working Solution

1. Add 50 µL of ddH_2O to the vial of Buccutite™ FOL-Activated PE-iFluor® 710 (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-iFluor® 710 Conjugation

1. Add 50 µL of Buccutite™ FOL-Activated PE-iFluor® 710 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-iFluor® 710 conjugate is now ready to use.

Note: For immediate use, the antibody-PE-iFluor® 710 conjugate must be diluted with the buffer of your choice.

Note: For longer term storage, antibody-PE-iFluor® 710 conjugate solution must be concentrated or freeze dried.

Storage of Antibody-PE-iFluor® 710 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-iFluor® 710 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)
1325	PerCP	482	677
1310	PE	565	575
1318	PE-Texas Red	565	600
1356	PE-iFluor® 594	565	606
1322	PE-Cy5	565	674
1316	PE-Cy5.5	565	700
1358	PE-iFluor® 710	565	710
1317	PE-Cy7	565	780
1311	APC	651	662
1320	APC-Cy5.5	651	700
1319	APC-iFluor® 700	651	713
1321	APC-Cy7	651	780

EXAMPLE DATA ANALYSIS AND FIGURES

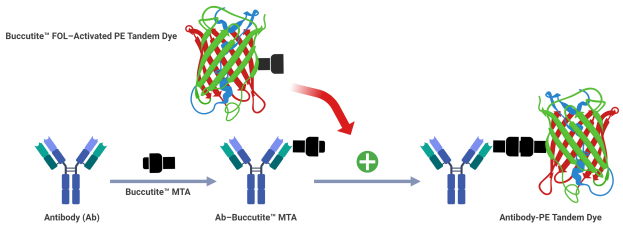

Figure 1. AAT Bioquest offers the Buccutite™ rapid labeling kit to streamline PE tandem dye conjugation for antibodies and other proteins, including streptavidin and secondary reagents. This kit utilizes preactivated PE modified with Buccutite™ FOL, while your antibody or protein is modified with Buccutite™ MTA to produce MTA-modified proteins. The MTA-modified proteins react efficiently with FOL-modified PE, yielding the desired PE-antibody conjugate with significantly higher efficiency compared to traditional SMCC chemistry. Additionally, the reaction requires much lower biopolymer concentrations, enhancing efficiency and reducing material usage compared to SMCC-based methods.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.