Buccutite™ Rapid PE-Cy7 Tandem IGM Antibody Labeling Kit *Optimized for Labeling 100 ug Antibody Per Reaction*

Catalog number: 79057
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 79057)
Component A: Buccutite™ FOL-Activated PE-Cy7	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

Buccutite™ Rapid PE-Cy7 Tandem IgM Antibody Labeling Kit provides a convenient method for conjugating IgM antibodies with PE-Cy7, a tandem fluorophore composed of R-Phycoerythrin (PE) donor and Cy7 acceptor. PE exhibits excitation/emission maxima of ~565/574 nm and transfers energy efficiently to Cy7, resulting in near-infrared emission suitable for multicolor flow cytometry applications. The kit can also be used for labeling other proteins such as streptavidin and secondary reagents. Buccutite™ Rapid PE-Cy7 Tandem IgM Antibody Labeling Kit includes preactivated PE-Cy7 and reaction buffer, enabling a simple labeling workflow consisting of two mixing steps without the need for additional purification. Considering the large molecular size of PE (~240 kDa), the amount of antibody used in a labeling reaction should be less than the amount of PE-Cy7, and optimal labeling ratios may be determined experimentally. A typical starting ratio is 100 µg of antibody for every 200 µg of PE-Cy7. Buccutite™ chemistry enables conjugation through amine groups that are abundant in proteins, avoiding the need for antibody reduction steps required in conventional SMCC-based thiol conjugation methods. Each kit supports two labeling reactions, each for up to 100 µg of antibody. The resulting PE-Cy7-labeled conjugates are suitable for applications including flow cytometry and other fluorescence-based detection workflows.

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 PE-Cy7 (Component A)
5. Incubate at room temperature for 60 minutes

Important Note

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 PE-Cy7 (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

IGM 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 ReadiUse™ 10KD Spin Filter (Cat. # 60502 from AAT Bioquest) 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 IGM 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 IGM antibody-PE-Cy7 conjugation

1. Make Buccutite™ FOL-Activated PE-Cy7 solution by adding 50 µL ddH2O into the vial of Buccutite™ FOL-Activated PE-Cy7 (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-Cy7 solution into the antibody-Buccutite™ MTA solution, mix well and rotating the mixture for 1 hour at room temperature.

3. The antibody-PE-Cy7 conjugate is now ready to use.

   Note: For immediate use, the antibody-PE-Cy7 conjugate need be diluted with the buffer of your choice.

Storage of IGM Antibody-PE-Cy7 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-PE-Cy7 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-Cy7 conjugates could be lyophilized and stored at ≤ –20 °C.

DISCLAIMER

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