Buccutite™ Rapid PE IgM Antibody Labeling Kit *Optimized for Labeling 25 ug Antibody Per Reaction*

Catalog number: 79050
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 79050)
Component A: Buccutite™ FOL-Activated PE	Minimize light exposure, Refrigerated (2-8 °C)	2 vials
Component B: Buccutite™ MTA	Minimize light exposure, Refrigerated (2-8 °C)	2 vials
Component C: Reaction Buffer	Minimize light exposure, Refrigerated (2-8 °C)	1 vial (20 µL)

OVERVIEW

Buccutite™ Rapid PE IgM Antibody Labeling Kit (microscale) provides a convenient method for conjugating IgM antibodies with R-Phycoerythrin (PE), an orange fluorescent protein with an excitation wavelength of 565 nm and an emission wavelength of 574 nm. The kit can also be used for labeling other proteins such as streptavidin and secondary reagents. Buccutite™ Rapid PE IgM Antibody Labeling Kit includes preactivated PE 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, and optimal labeling ratios may be determined experimentally. A typical starting ratio is 25 µg of antibody for every 50 µg of PE. 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 25 µg of antibody. The resulting PE-labeled conjugates are suitable for applications including flow cytometry and other fluorescence-based detection workflows.

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 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. TThis protocol is provided as a general recommendation.

PREPARATION OF WORKING SOLUTION

IgM 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 PE working solution

Add 50 µL ddH_2O into the vial of Buccutite ™ FOL-Activated PE (Component A).

SAMPLE EXPERIMENTAL PROTOCOL

Run IgM 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 conjugation

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

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

   Note: For longer term storage, antibody-PE conjugate solution need be concentrated or freeze dried.

Storage of Antibody-PE 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 conjugate solution could be stored at 4 °C for two

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months without significant change and kept from light.

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