# Buccutite™ Rapid PE IgM Antibody Labeling Kit *Optimized for Labeling 100 ug Antibody Per Reaction*

Catalog number: 79055
Unit size: 2 Labelings

| Component | Storage | Amount (Cat No. 79055) |
|---|---|---|
| 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 (100 µg scale) 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 50–60 µg of antibody for every 100 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 100 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 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 (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 (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. This protocol is provided as a general recommendation.

## 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 antibody-PE conjugation

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

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

**Table 1.** Available fluorophores at AAT Bioquest Buccutite™ Rapid Antibody Labelling Kits

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