

Buccutite™ Rapid APC IGM Antibody Labeling Kit *Optimized for Labeling 100 ug Antibody Per Reaction*

Catalog number: 79065
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 79065)
Component A: Buccutite™ FOL-Activated APC	Refrigerated (2-8 °C), Minimize light exposure	2 vials
Component B: Buccutite™ MTA	Refrigerated (2-8 °C), Minimize light exposure	2 vials
Component C: Reaction Buffer	Refrigerated (2-8 °C), Minimize light exposure	1 Vial (20 µL)

OVERVIEW

Buccutite™ Rapid APC IgM Antibody Labeling Kit provides a convenient method for conjugating IgM antibodies with APC, a red fluorescent protein with excitation and emission maxima of ~651 nm and ~662 nm. AAT Bioquest offers this Buccutite™ rapid labeling kit to facilitate APC conjugation to antibodies and other proteins such as streptavidin and secondary reagents. The kit includes preactivated APC and reaction buffer, enabling a simple labeling workflow consisting of two mixing steps without the need for additional purification. Each kit supports two labeling reactions, each for up to 100 µg of antibody. 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. The resulting APC-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 IGM 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 APC (Component A)
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, Component B can be stored at -20°C. Do not freeze Buccutite™ FOL-Activated APC (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-APC conjugation

1. Make Buccutite™ FOL-Activated APC solution by adding 50 µL ddH₂O into the vial of Buccutite™ FOL-Activated APC (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 APC solution into the antibody-Buccutite™ MTA solution, mix well and rotating the mixture for 1 hour at room temperature.
3. The antibody-APC conjugate is now ready to use.

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

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

DISCLAIMER

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have any questions.