

Buccutite™ Rapid APC-iFluor™ 700 Tandem IGM Antibody Labeling Kit *Optimized for Labeling 100 ug Antibody Per Reaction*

Catalog number: 79066
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 79066)
Component A: Buccutite™ FOL-Activated APC-iFluor® 700	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-iFluor™ 700 Tandem IgM Antibody Labeling Kit provides a convenient method for conjugating IgM antibodies with APC-iFluor™ 700, a tandem fluorophore composed of APC donor and iFluor™ 700 acceptor. APC exhibits excitation/emission maxima of ~651/662 nm and transfers energy efficiently to iFluor™ 700, resulting in far-red emission (~710 nm) suitable for multicolor flow cytometry applications. The kit can also be used for labeling other proteins such as streptavidin and secondary reagents. Buccutite™ Rapid APC-iFluor™ 700 Tandem IgM Antibody Labeling Kit includes preactivated APC-iFluor™ 700 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-iFluor™ 700-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-iFluor® 700 (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-iFluor® 700 (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-iFluor® 700 conjugation

1. Make Buccutite™ FOL-Activated APC-iFluor® 700 solution by adding 50 µL ddH2O into the vial of Buccutite™ FOL-Activated APC-iFluor® 700 (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-iFluor® 700 solution into the antibody-Buccutite™ MTA solution, mix well and rotating the mixture for 1 hour at room temperature.
3. The antibody-APC-iFluor® 700 conjugate is now ready to use.

Note: For immediate use, the antibody-APC-iFluor® 700 conjugate need be diluted with the buffer of your choice.

Storage of IgM Antibody-APC-iFluor® 700 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-iFluor® 700 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-iFluor® 700 conjugates could be lyophilized and stored at ≤ -20 °C.

DISCLAIMER

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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.