BTC, AM *CAS 176767-94-5*

Catalog number: 21054
Unit size: 1 mg

Component	Storage	Amount
BTC, AM *CAS 176767-94-5*	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

OVERVIEW

This cell-permeant coumarin Ca2+ indicator BTC, AM exhibits a shift in excitation maximum from about 480 nm to 400 nm upon binding Ca2+, enabling ratiometric calcium measurements. Due to its high selectivity and low affinity for Ca2+ (Kd ~7 uM) BTC is often used for the quantitation of high intracellular Ca2+levels. In addition, BTC, AM has also been used for monitoring potassium channel since thallium ion enhances the fluorescence of BTC.

KEY PARAMETERS

Fluorescence microscope

Excitation	FITC filter set
Emission	FITC filter set
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Excitation	400, 480
Emission	540
Cutoff	515
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Bottom read mode

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

BTC AM Stock Solution
Prepare a 2 to 5 mM stock solution of BTC AM in high-quality, anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

BTC AM Working Solution
On the day of the experiment, either dissolve BTC AM in DMSO or thaw an aliquot of the indicator stock solution to room temperature. Prepare a dye working solution of 2 to 20 µM in a buffer of your choice (e.g., Hanks and Hepes buffer) with 0.04% Pluronic® F-127. For most cell lines, BTC AM at a final concentration of 4 to 5 µM is recommended. The exact concentration of indicators required for cell loading must be determined empirically.

Note The nonionic detergent Pluronic® F-127 is sometimes used to increase the aqueous solubility of BTC AM. A variety of Pluronic® F-127 solutions can be purchased from AAT Bioquest.

Note If your cells contain organic anion-transporters, probenecid (1-2 mM) may be added to the dye working solution (final in well concentration will be 0.5-1 mM) to reduce leakage of the de-esterified indicators. A variety of ReadiUse™ probenecid products, including water-soluble, sodium salt, and stabilized solution, can be purchased from AAT Bioquest.

SAMPLE EXPERIMENTAL PROTOCOL

Following is our recommended protocol for loading AM esters into live cells. This protocol only provides a guideline and should be modified according to your specific needs.

1. Prepare cells in growth medium overnight.
2. On the next day, add 1X BTC AM working solution into your cell plate.

   Note If your compound(s) interfere with the serum, replace the growth medium with fresh HHBS buffer before dye-loading.
3. Incubate the dye-loaded plate in a cell incubator at 37 °C for 30 to 60 minutes.

   Note Incubating the dye for longer than 1 hour can improve signal intensities in certain cell lines.
4. Replace the dye working solution with HHBS or buffer of your choice (containing an anion transporter inhibitor, such as 1 mM probenecid, if applicable) to remove any excess probes.
5. Add the stimulant as desired and simultaneously measure fluorescence using either a fluorescence microscope equipped with a FITC filter set or a fluorescence plate reader containing a programmable liquid handling system such as a FlexStation, at Ex/Em $_1$ = 400/540 nm cutoff 515 nm and Ex/Em $_2$ = 480/540 nm cutoff 515 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

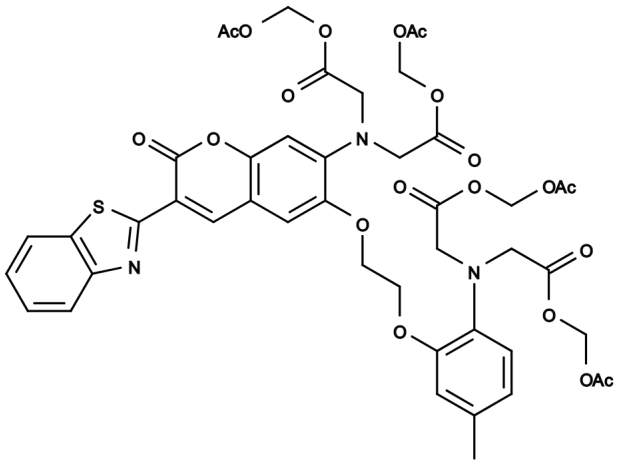

Figure 1. Chemical structure for BTC, AM *CAS 176767-94-5*

DISCLAIMER

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