Cal Green™ 1, AM [Equivalent to Calcium Green-1, AM]

Catalog number: 20501, 20502
Unit size: 10x50 ug, 1 mg

Component	Storage	Amount (Cat No. 20501)	Amount (Cat No. 20502)
Cal Green™ 1, AM [Equivalent to Calcium Green -1, AM]	Freeze (< -15 °C), Minimize light exposure	10x50 ug	1 vial (1 mg)

OVERVIEW

Cal Green™ 1 (AAT Bioquest) is the same molecule to Calcium Green-1 (Invitrogen). It exhibits an increase in fluorescence intensity upon binding calcium ion. The cell-permeant dye, Cal Green 1 AM (Calcium Green-1 AM) is a 488 nm-excitable calcium indicator. Compared to Fluo-3 AM, Cal Green-1 AM is more fluorescent at low calcium concentrations in cells, facilitating the determination of baseline calcium levels and increasing the visibility of resting cells. It has been used in many calcium signaling investigations, including measuring intracellular calcium, following calcium influx and release, and multiphoton excitation imaging of calcium in living tissues. Cells can be loaded with Cal Green-1 AM by adding the dissolved indicator directly to cultured cells in medium. The fluorescence signal from these cells is generally measured using fluorescence microscopy, fluorescence microplate assays, or flow cytometry.

KEY PARAMETERS

Fluorescence microscope

Emission	FITC
Excitation	FITC
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Cutoff	515
Emission	525
Excitation	490
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Bottom read mode/Programmable liquid handling

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

Cal Green™ 1 AM Stock Solution

1. Prepare a 2 to 5 mM stock solution of Cal Green™ 1 AM in high-quality, anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

Cal Green™ 1 AM Working Solution

1. On the day of the experiment, either dissolve Cal Green™ 1 AM in DMSO or thaw an aliquot of the indicator stock solution to room temperature.

2. Prepare a 2 to 20 µM Cal Green™ 1 AM working solution in a buffer of your choice (e.g., Hanks and Hepes buffer) with 0.04% Pluronic® F-127. For most cell lines, Cal Green™ 1 AM at a final concentration of 4-5 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 Cal Green™ 1 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 solutions, 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 Cal Green™ 1 AM working solution to 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 an FDSS, FLIPR, or FlexStation, at Ex/Em = 490/525 nm cutoff 515 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

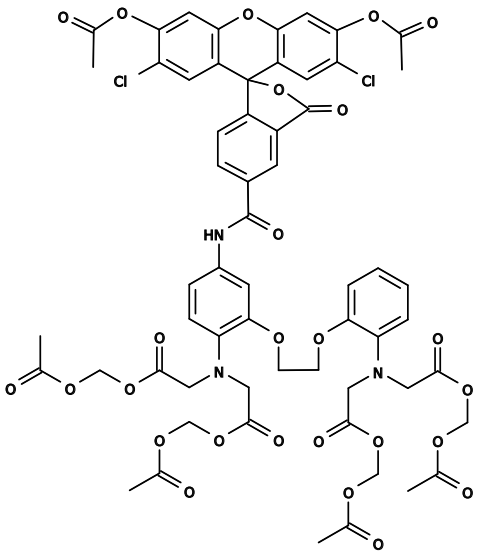

Figure 1. Chemical structure for Cal Green™ 1, AM [Equivalent to Calcium Green-1, AM]

DISCLAIMER

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