Calbryte™ 590 AM

Catalog number: 20700, 20701, 20702
Unit size: 2x50 ug, 10x50 ug, 1 mg

Component	Storage	Amount (Cat No. 20700)	Amount (Cat No. 20701)	Amount (Cat No. 20702)
Calbryte™ 590 AM	Freeze (< -15 °C), Minimize light exposure	2x50 ug	10x50 ug	1 vial (1 mg)

OVERVIEW

The intracellular calcium flux assay is a widely used method in monitoring signal transduction pathways and high throughput screening of G protein"coupled receptors (GPCRs) and calcium channel targets. Followed by Rhod-2 being introduced in 1989, Rhod-4 and Cal-590 were later developed with improved signal/background ratio, and they became the widely used red fluorescent Ca2+ indicators for confocal microscopy, flow cytometry and high throughput screening applications. In CHO and HEK cells Rhod-4 and Cal-590 have cellular calcium response that are 10 times more sensitive than Rhod-2 AM. However, Cal-590 and Rhod-4 are still less sensitive to calcium in cells than the corresponding green fluorescent calcium indicators (e.g., Fluo-8 and Cal-520). Calbryte™ 590 is a new generation of red fluorescent indicators for the measurement of intracellular calcium. Its greatly improved signal/background ratio and intracellular retention properties make Calbryte™ 590 AM the most robust red fluorescent indicator for evaluating GPCR and calcium channel targets as well as for screening their agonists and antagonists in live cells. Like other dye AM cell loading, Calbryte™ 590 AM ester is non-fluorescent and once gets inside the cell, it is hydrolyzed by intracellular esterase and gets activated. The activated indicator is a polar molecule that is no longer capable of freely diffusing through cell membrane, essentially trapped inside cells.

KEY PARAMETERS

Fluorescence microscope

Emission	TRITC/Cy3
Excitation	TRITC/Cy3
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Cutoff	570
Emission	590
Excitation	540
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

Calbryte™ 590 AM Stock Solution

1. Prepare a 2 to 5 mM stock solution of Calbryte™ 590 AM in anhydrous DMSO.

   Note: When reconstituted in DMSO, Calbryte™ 590 AM is a clear, colorless solution.

PREPARATION OF WORKING SOLUTION

Calbryte™ 590 AM Working Solution

1. On the day of the experiment, either dissolve Calbryte™ 590 AM in DMSO or thaw an aliquot of the indicator stock solution to room temperature.

2. Prepare a 2 to 20 µM Calbryte™ 590 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, Calbryte™ 590 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 Calbryte™ 590 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 Calbryte™ 590 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 TRITC/Cy3 filter set or a fluorescence plate reader containing a programmable liquid handling system such as an FDSS, FLIPR, or FlexStation, at Ex/Em = 540/590 nm cutoff 570 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

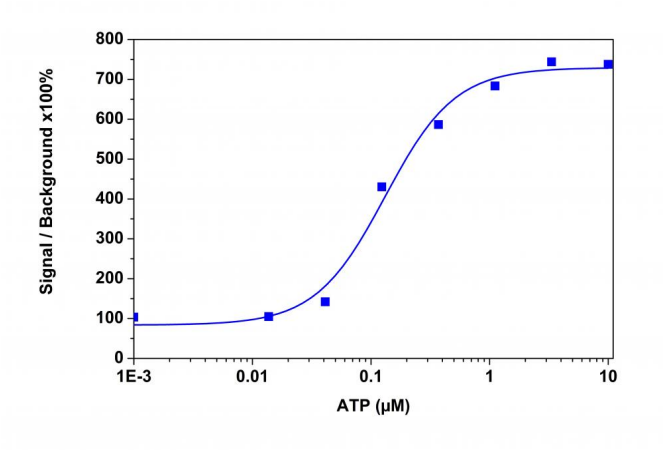

Figure 1. An ATP dose-response was measured in CHO-K1 cells with Calbryte™ 590 AM. CHO-K1 cells were seeded overnight at 50,000 cells/100 µL/well in a 96-well black wall/clear bottom costar plate. 100 µL of 10 µg/ml Calbryte™ 590 AM in HH Buffer with probenecid was added and incubated for 60 min at 37°C. Dye loading solution was then removed and replaced with 200 µL HH Buffer/well. ATP (50 µL/well) was added by FlexStation 3 to achieve the final indicated concentrations.

DISCLAIMER

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