Calbryte™ 520 AM

Catalog number: 20650, 20651, 20653
Unit size: 2x50 ug, 10x50 ug, 1 mg

Component	Storage	Amount (Cat No. 20650)	Amount (Cat No. 20651)	Amount (Cat No. 20653)
Calbryte™ 520 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 Fluo-3 being introduced in 1989, Fluo-4, Fluo-8 and Cal-520 were later developed with improved signal/background ratio, and became the widely used Ca2+ indicators for confocal microscopy, flow cytometry and high throughput screening applications. However, there are still a few severe problems with Fluo-4. For example, as for Fluo-3, in all most all the intracellular calcium assays with Fluo-4 AM, probenecid is required to prevent the cell-loaded Fluo-4 from leaking out of cells. The use of probenecid with Fluo-4-based calcium assays compromises the assay results since probenecid is well-documented to have a variety of complicated cellular effects. Calbryte™ 520, AM is a novel fluorescent and cell-permeable indicator for the measurement of intracellular calcium. Like other dye AM esters, Calbryte™ 520 AM is non-fluorescent and non-activatable. Once Calbryte™ 520 AM enters the cell, it is readily hydrolyzed by intracellular esterase where it becomes activated and responsive to calcium. The activated indicator is now a polar molecule that is incapable of freely diffusing through the cell membrane, essentially trapping it inside the cell. Upon binding calcium ions, Calbryte™ 520 produces a bright fluorescence signal with extremely high signal/background ratio. It has the identical excitation and emission wavelength as Fluo-4, thus the same Fluo-4 assay settings can be readily applied to Calbryte™ 520-based calcium assays. Its greatly improved signal/background ratio and intracellular retention properties make Calbryte™ 520 AM the most robust indicator for evaluating GPCR and calcium channel targets as well as for screening their agonists and antagonists in live cells.

KEY PARAMETERS

Flow cytometer

Excitation	488 nm laser
Emission	530/30 nm filter
Instrument specification(s)	FITC channel

Fluorescence microscope

Excitation	FITC
Emission	FITC
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Excitation	490
Emission	525
Cutoff	515
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™ 520 AM Stock Solution
Prepare a 2 to 5 mM stock solution of Calbryte™ 520 AM in anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

Calbryte™ 520 AM Working Solution
On the day of the experiment, either dissolve Calbryte™ 520 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, Calbryte™ 520 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 Calbryte™ 520 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 Calbryte™ 520 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 an FDSS, FLIPR, or FlexStation, at Ex/Em = 490/525 nm cutoff 515 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

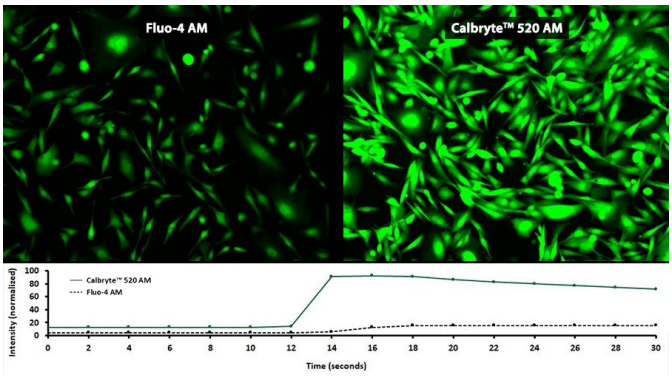

Figure 1. ATP response was measured in CHO-K1 cells using Calbryte™ 520 AM (Cat No. 20653) and Fluo-4, AM (Cat No. 20550). CHO-K1 cells were seeded overnight at 50,000 cells/100 µL/well in a 96-well black wall/clear bottom costar plate. 100 µL of either 10 µg/mL Calbryte™ 520 AM in HH Buffer with probenecid or 10 µg/mL Fluo-4, AM in HH Buffer with probenecid was added to the wells and incubated for 45 minutes at 37°C. Both dye loading solutions were removed and replaced with 200 µL HH Buffer/well. ATP (50 µL/well) was added to achieve the final indicated concentration of 10 µM. Images were acquired on a Keyence microscope in the FITC channel.

DISCLAIMER

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