Calbryte™-520L AM

Catalog number: 20640
Unit size: 10x50 ug

Component	Storage	Amount (Cat No. 20640)
Calbryte™-520L AM	Freeze (< -15 °C), Minimize light exposure	10x50 ug

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. Following the introduction of Fluo-3 in 1989, Fluo-4, Fluo-8, and Cal-520 were later developed with enhanced signal-to-background ratios and soon became the Ca2+ indicators of choice for confocal microscopy, flow cytometry, and high throughput screening applications. However, there are still a few caveats with Fluo-4. For example, like Fluo-3, Fluo-4 exhibits poor intracellular retention, and the use of 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 520L, AM is a new fluorescent and cell-permeable calcium indicator. Like other dye AM cell loading, Calbryte 520L AM ester is non-fluorescent, and once it 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 the cell membrane and is essentially trapped inside cells. Calbryte 520L has a low affinity to calcium ions with a Kd ~ 91 μM. Calbryte 520L produces a bright fluorescence signal in the presence of calcium at high concentrations. It has the identical excitation and emission wavelength as Fluo-4; thus, the same Fluo-4 assay settings can be readily applied to Calbryte 520L-based calcium assays.

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

Calbryte™-520L AM Stock Solution

1. Prepare a 2 to 5 mM stock solution of Calbryte™-520L AM in anhydrous DMSO.

   Note: When reconstituted in DMSO, Calbryte™-520L AM is a clear, colorless solution.

PREPARATION OF WORKING SOLUTION

Calbryte™-520L AM Working Solution

1. On the day of the experiment, either dissolve Calbryte™-520L AM in DMSO or thaw an aliquot of the indicator stock solution to room temperature.

2. Prepare a 2 to 20 μM Calbryte™-520L 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™-520L 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™-520L 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™-520L 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

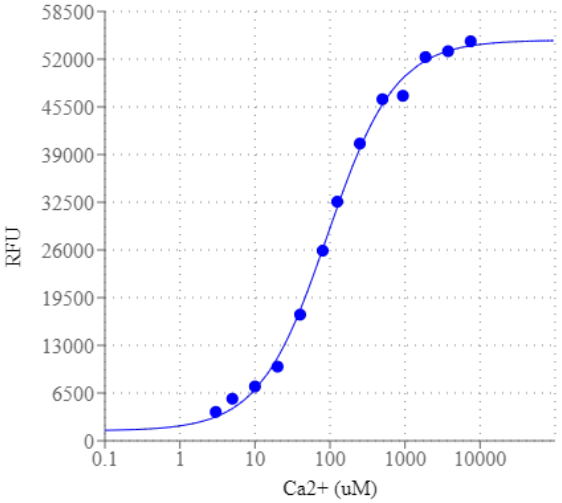

Figure 1. Ca2+ Dependent Dose Response of Calbryte™-520L AM.

DISCLAIMER

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