Calbryte™ 630 AM

Catalog number: 20720, 20721, 20722
Unit size: 2x50 ug, 10x50 ug, 1 mg

Component	Storage	Amount (Cat No. 20720)	Amount (Cat No. 20721)	Amount (Cat No. 20722)
Calbryte™ 630 AM	Freeze (< -15 °C), Minimize light exposure	2x50 ug	10x50 ug	1 vial (1 mg)

OVERVIEW

Calcium measurement is critical for numerous biological investigations. Fluorescent probes that show spectral responses upon binding calcium have enabled researchers to investigate changes in intracellular free calcium concentrations by using fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers. x-Rhod-1 is commonly used as a red fluorescent calcium indicator. However, x-Rhod-1 is only moderately fluorescent in live cells upon esterase hydrolysis, and has very small cellular calcium responses. Calbryte™ 630 has been developed to improve x-Rhod-1 cell loading and calcium response while maintaining the spectral wavelength of x-Rhod-1, making it compatible with Texas Red® filter set. In CHO and HEK cells Cal-630™ AM has cellular calcium response that is much more sensitive than x-Rhod-1. The spectra of Calbryte™ 630 is well separated from those of FITC, Alexa Fluor® 488 and GFP, making it an ideal calcium probe for multiplexing intracellular assays with GFP cell lines or FITC/Alexa Fluor® 488 labeled antibodies. Calbryte™ 630 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™ 630 AM the most robust deep 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™ 630 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

Flow cytometer

Excitation	640 nm laser
Emission	660/20 nm filter
Instrument specification(s)	APC channel

Fluorescence microscope

Excitation	Texas Red
Emission	Texas Red
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Excitation	600
Emission	640
Cutoff	630
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™ 630 AM Stock Solution
Prepare a 2 to 5 mM stock solution of Calbryte™ 630 AM in anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

Calbryte™ 630 AM Working Solution

On the day of the experiment, either dissolve Calbryte™ 630 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™ 630 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™ 630 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™ 630 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 Texas Red filter set or a fluorescence plate reader containing a programmable liquid handling system such as an FDSS, FLIPR, or FlexStation, at Ex/Em = 600/640 nm cutoff 630 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

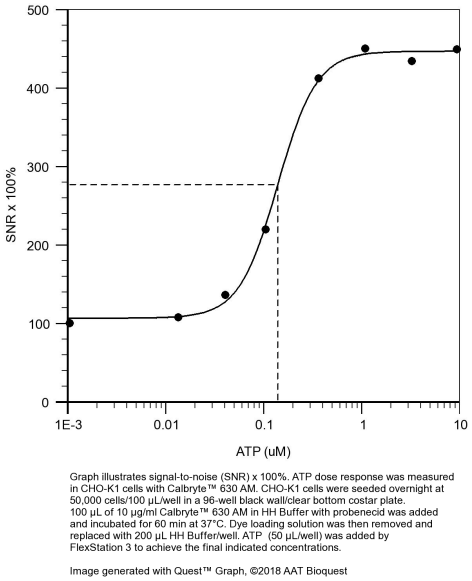

Figure 1. Graph illustrates signal-to-noise (SNR) x 100%. ATP dose response was measured in CHO-K1 cells with Calbryte™ 630 AM. CHO-K1 cells were seeded overnight at 50,000 cells/100 µL/well in a 96-well black wall/clear bottom costar plate. 100 µL of 10 µg/ml Calbryte™ 630 AM in HH Buffer with probenecid 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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