Calbryte™ 520, Calbryte™ 590 and Calbryte™ 630 Calcium Detection Reagents

Introduction

Calbryte™ 520, Calbryte™ 590 and Calbryte™ 630 provide the most robust homogeneous fluorescence-based assay tools for detecting intracellular calcium mobilization. These calcium-sensitive dyes are significantly brighter, and provide much higher signal to noise ratio with greatly improved intracellular retention and loading efficiency compared to the existing calcium indicators (such as Fluo-3 AM, Fluo-4 AM and Rhod-2 AM). Cells expressing a GPCR or calcium channel of interest that signals through calcium can be preloaded with Calbryte™ 520 AM, Calbryte™ 590 AM or Calbryte™ 630 AM which can cross cell membranes. Once inside cells, the lipophilic blocking AM groups of Cal 520® AM, Calbryte™ 590 AM or Calbryte™ 630 AM are cleaved by intracellular esterases, resulting in a negatively charged fluorescent Calbryte™ dyes that stays inside cells. Their fluorescence is greatly enhanced upon binding to calcium. When cells stimulated with agonists, the receptor signals the release of intracellular calcium, which significantly increase the fluorescence of Calbryte™ 520, Calbryte™ 590 or Calbryte™ 630. The characteristics of high sensitivity and >100 times fluorescence enhancement make Calbryte™ 520 AM, Calbryte™ 590 AM or Calbryte™ 630 AM the most robust indicators for measuring intracellular calcium.

Besides their convenient excitation wavelengths and large fluorescence enhancement by calcium, Calbryte™ 520, Calbryte™ 590, and Calbryte™ 630 are predominantly localized in cytosols unlike Rhod-2 that is mainly localized in mitochondria. In addition, the long Ex/Em wavelengths of Calbryte™ 590 and Calbryte™ 630 make these dyes perfect calcium indicators compatible for multicolor detection with green fluorescent protein (GFP) cell lines. In addition, Calbryte™ 520, Calbryte™ 590 or Calbryte™ 630 calcium assays are optimized to be compatible with most of the existing fluorescence instruments. Calbryte™ 520 can be well excited at 488 nm, and used with FITC filter set. Calbryte™ 590 is optimized to be excited at 555 nm, and used with TRITC/Cy3 filter set. Calbryte™ 630 is optimized to be excited at 594 nm, and used with Texas Red® filter set. The spectral and calcium binding properties are summarized below (see Table 1).

Table 1. Spectral and Ca^{2+}–Binding Properties of Calbryte™ 520, Calbryte™ 590 or Calbryte™ 630 Reagents

Ca^{2+} Indicator	Excitation (nm)	Emission (nm)	K_d (µM)
Calbryte™ 520	492 nm	514 nm	1.2
Calbryte™ 590	580 nm	592 nm	1.4
Calbryte™ 630	608 nm	624 nm	1.2

Use of Calbryte™ 520 AM, Calbryte™ 590 AM, or Calbryte™ 630 AM Esters

1. Load Cells with Calbryte™ 520, Calbryte™ 590 or Calbryte™ 630 AM Esters:

Calbryte™ AM esters should be reconstituted just before use in anhydrous DMSO. The DMSO stock solutions may be stored (desiccated) at –20 °C and protected from light. Under these conditions, AM esters should be stable for three months. Following is our recommended protocol for loading Calbryte™ 520 AM, Calbryte™ 590 AM or Calbryte™ 630 AM esters into live cells. This protocol only provides a guideline, and should be modified according to your specific needs.

a) Prepare a 2 to 5 mM stock solution of Calbryte™ 520 AM, Calbryte™ 590 AM or Calbryte™ 630 AM esters in anhydrous DMSO.

b) Dissolve Calbryte™ 520 AM, Calbryte™ 590 AM or Calbryte™ 630 AM in DMSO or thaw an aliquot of the indicator stock solution to room temperature. Prepare a dye working solution of 10 to 20 µM in Hanks and Hepes buffer (HHBS) or the buffer of your choice with 0.04% Pluronic® F-127. The exact concentration of the indicator 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, Calbryte™ 590 AM or Calbryte™ 630 AM esters. A variety of Pluronic® F-127 solutions can be purchased from AAT Bioquest.

c) If your cells (such as CHO cells) contain organic anion-transports, 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. Probenecid might not be needed even in CHO cells with Calbryte™ 520 AM.
Note: A variety of ReadiUse™ probenecid including water soluble sodium salt and stabilized solution can be purchased from AAT Bioquest.

d) Add equal volume of the dye working solution (from Step b or c) into your cell plate.

e) Incubate the dye-loading plate in a cell incubator for ~60 minutes, and then incubate the plate at room temperature for another 15 minutes.

f) Replace the dye working solution with HHBS or a buffer of your choice that contains an anion transporter inhibitor, such as 1 mM probenecid, to remove excess probes.

g) Run the calcium tests at Ex/Em = 490/525 nm for Calbryte™ 520 AM, 540/590 nm for Calbryte™ 590 AM or 610/640 nm for Calbryte™ 630 AM.

2. Measure Intracellular Calcium Responses:

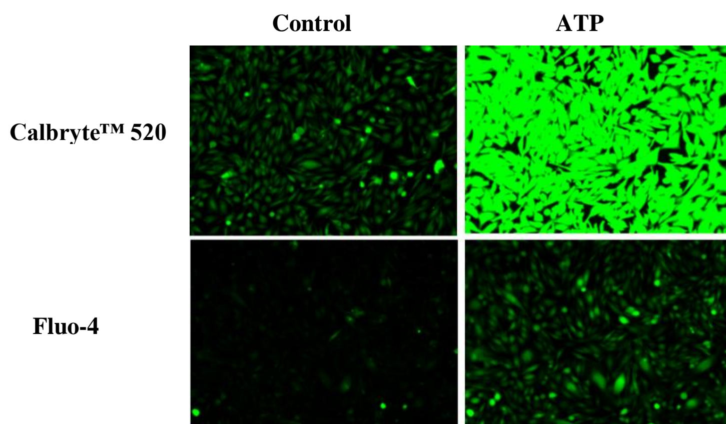

Figure 1. Response of endogenous P2Y receptor to ATP in CHO-K1 cells. CHO-K1 cells were seeded overnight at 40,000 cells per 100 µL per well in a 96-well black wall/clear bottom costar plate. 100 µL of Fluo-4 AM or Calbryte™ 520 AM in HHBS with probenecid were added into the wells, and the cells were incubated at 37 °C for 45min. The dye loading medium were replaced with 200 µL HHBS, 50 µL of 50 µM ATP were added, and imaged with a fluorescence microscope (Keyence) using FITC channel.

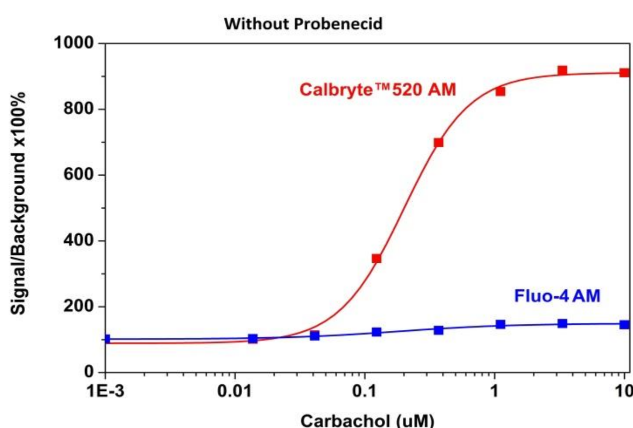

Figure 2. Carbachol-stimulated calcium response of exogenous M1 receptor in CHO-M1 cells measured with Calbryte™ 520 or Fluo-4 AM. CHO-M1cells were seeded overnight in 40,000 cells per 100 µL per well in a 96-well black wall/clear bottom costar plate. 100 µLof Fluo-4 AM or the Calbryte™ 520 AM without probenecid was added into the cells, and the cells were incubated at 37 °C for 45min. Carbachol (50 µL/well) was added by FlexStation 3 (Molecular Devices) to achieve the final indicated concentrations.

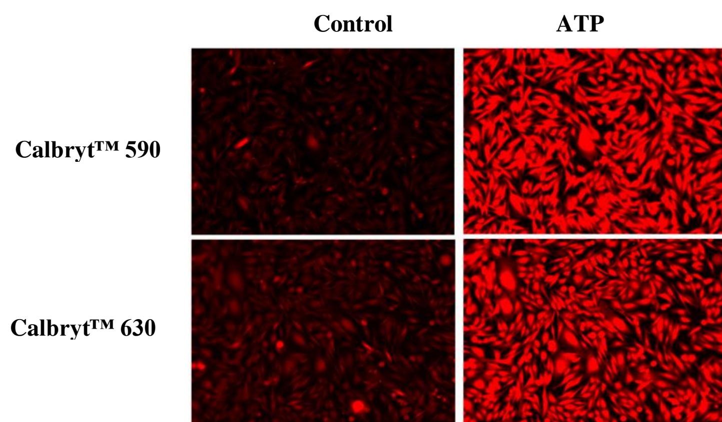

Figure 3. Response of endogenous P2Y receptor to ATP in CHO-K cells. CHO-K cells were seeded overnight at 40,000 cells per 100 µL per well in a 96-well black wall/clear bottom costar plate. 100 µL of Calbryte™ 590 AM or Calbryte™ 630 AM in HHBS with 2 mM probenecid were added into the wells, and the cells were incubated at 37 °C for one hour. The dye loading mediums were replaced with 200 µL HHBS, treated with 50 µL of 50 µM ATP, and imaged with a fluorescence microscope (Keyence) using TRITC channel (Calbryte™ 590) and Texas Red Channel (Calbryte™ 630).

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Use of Calbryte™ 520, Calbryte™ 590 or Calbryte™ 630 Salts

Calcium calibration can be carried out by measuring the fluorescence intensity of the salt form (25 to 50 µM in fluorescence microplate readers) of the indicators in solutions with precisely known free Ca^{2+} concentrations. Calibration solutions can be used based on 30 mM MOPS EGTA Ca^{2+} buffer. In general, water contains trace amount of calcium ion. It is highly recommended to use 30 mM MOPS + 100 mM KCl, pH 7.2 as buffer system. One can simply make a 0 and 39 µM calcium stock solutions as listed below, and these 2 solutions are used to make a serial solutions of different Ca^{2+} concentrations

A. 0 µM calcium: 30 mM MOPS + 100 mM KCl, pH 7.2 buffer + 10 mM EGTA
B. 39 µM calcium: 30 mM MOPS + 100 mM KCl, pH 7.2 buffer + 10 mM EGTA + 10 mM $CaCl_2$

To determine either the free calcium concentration of a solution or the K_d of a single-wavelength calcium indicator, equation $[Ca]_{free} = K_d[F - F_{min}]/[F_{max} - F]$ is used.

Where F is the fluorescence intensity of the indicator at a specific experimental calcium level, F_{min} is the fluorescence intensity in the absence of calcium and F_{max} is the fluorescence intensity of the calcium-saturated probe.

The dissociation constant (K_d) is a measure of the affinity of the probe for calcium. The calcium-binding and spectroscopic properties of fluorescent indicators vary quite significantly in cellular environments compared to calibration solutions. *In situ* response calibrations of intracellular indicators typically yield K_d values significantly higher than *in vitro* determinations. *In situ* calibrations are performed by exposing loaded cells to controlled Ca^{2+} buffers in the presence of ionophores such as A-23187, 4-bromo A-23187 and ionomycin. Alternatively, cell permeabilization agents such as digitonin or Triton® X-100 can be used to expose the indicator to the controlled Ca^{2+} levels of the extracellular medium.

Conclusions

Because of the importance of Ca^{2+} in biology, numerous techniques/methods for analyzing the mechanisms of cellular and/or subcellular Ca^{2+} activity have been established. Although each method for analyzing Ca^{2+}activity has certain advantages over the others, each also suffers from drawbacks. With the outstanding properties described above, we believe that Calbryte™ 520, Calbryte™ 590 and Calbryte™ 630 calcium detection reagents provide a new powerful tool for intracellular calcium analysis and monitor in a variety of biological systems.

As might have been predicted, the interests of many researchers in Ca^{2+} analysis shifted from the cellular level to the subcellular level. It has been found that Ca^{2+} is not even distributed throughout the whole cell and that intracellular heterogeneity of Ca^{2+} (such as Ca^{2+} waves and Ca^{2+} sparks) is observed in a variety of cells (e.g., oocyte, heart muscle cell, hepatocyte, and exocrine cell). With the advent of the confocal laser scanning microscope (CLSM) in the 1980s and advanced microplate readers in 2000s (such as FLIPR, FDSS and NOVOStar dedicated for intracellular Ca^{2+} detections), the measurement of intracellular Ca^{2+} has accelerated significantly. Confocal laser scanning microscopy and more recently multiphoton microscopy allow the precise spatial and temporal analysis of intracellular Ca^{2+} signaling at the subcellular level in addition to the measurement of its concentration.

Product Ordering Information

Cat. #	Product Name	Unit Size
20650	Calbryte™ 520 AM *Cell-permeable*	2x50 µg
20651	Calbryte™ 520 AM *Cell-permeable*	10x50 µg
20653	Calbryte™ 520 AM *Cell-permeable*	1 mg
20656	Calbryte™ 520 potassium salt	2x50 µg
20658	Calbryte™ 520 potassium salt	10x50 µg
20700	Calbryte™ 590 AM *Cell-permeable*	2x50 µg
20701	Calbryte™ 590 AM *Cell-permeable*	10x50 µg
20702	Calbryte™ 590 AM *Cell-permeable*	1 mg
20706	Calbryte™ 590 potassium salt	5x50 µg
20720	Calbryte™ 630 AM *Cell-permeable*	2x50 µg
20721	Calbryte™ 630 AM *Cell-permeable*	10x50 µg
20722	Calbryte™ 630 AM *Cell-permeable*	1 mg

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20727	Calbryte™ 630 potassium salt	5x50 µg
36317	Screen Quest™ Calbryte 520 Probenecid-Free and Wash-Free Calcium Assay Kit	1 plate
36318	Screen Quest™ Calbryte 520 Probenecid-Free and Wash-Free Calcium Assay Kit	10 plates
36319	Screen Quest™ Calbryte 520 Probenecid-Free and Wash-Free Calcium Assay Kit	100 plates
36200	Screen Quest™ Calbryte 590 Probenecid-Free and Wash-Free Calcium Assay Kit	1 plate
36201	Screen Quest™ Calbryte 590 Probenecid-Free and Wash-Free Calcium Assay Kit	10 plates
36202	Screen Quest™ Calbryte 590 Probenecid-Free and Wash-Free Calcium Assay Kit	100 plates

References

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