Cal-520®, AM

Catalog number: 21130, 21131
Unit size: 10x50 ug, 1 mg

Component	Storage	Amount (Cat No. 21130)	Amount (Cat No. 21131)
Cal-520®, AM	Freeze (< -15 °C), Minimize light exposure	10x50 ug	1 vial (1 mg)

OVERVIEW

Cal-520® AM provides a robust homogeneous fluorescence-based assay tool for detecting intracellular calcium mobilization. Cal-520® AM is a new fluorogenic calcium-sensitive dye with a significantly improved signal to noise ratio and intracellular retention compared to the existing green calcium indicators (such as Fluo-3 AM and Fluo-4 AM). Cells expressing a GPCR or calcium channel of interest that signals through calcium can be preloaded with Cal-520® AM which can cross cell membrane. Once inside the cell, the lipophilic blocking groups of Cal-520™ AM are cleaved by esterases, resulting in a negatively charged fluorescent dye that stays inside cells. Its 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 Cal-520®. The characteristics of its long wavelength, high sensitivity, and >100 times fluorescence enhancement, make Cal-520® AM an ideal indicator for the measurement of cellular calcium. The high S/N ratio and better intracellular retention make the Cal-520® calcium assay a robust tool for evaluating GPCR and calcium channel targets as well as for screening their agonists and antagonists.

KEY PARAMETERS

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.

Cal-520® AM Stock Solution
Prepare a 2 to 5 mM stock solution of Cal-520® AM in high-quality, anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

Cal-520® AM Working Solution
On the day of the experiment, either dissolve Cal-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, Cal-520® 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 Cal-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 Cal-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 1 to 2 hours.

   Note Incubating the dye for longer than 2 hours 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

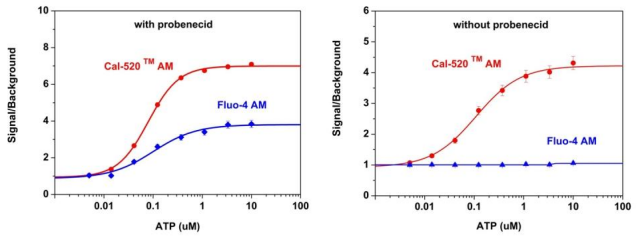

Figure 1. ATP-stimulated calcium responses of endogenous P2Y receptor in CHO-K1 cells incubated with Cal-520™ AM (red curve), or Fluo-4 AM (blue curve) respectively with (left) or without probenecid (right) under the same conditions. CHO-K1 cells were seeded overnight at 50,000 cells per 100 µL per well in a Costar black wall/clear bottom 96-well plate. 100 µL of 5 µM Fluo-4 AM or Cal 520™ AM in HHBS (with or without probenecid) was added into the cells, and the cells were incubated at 37 °C for 1 hour. ATP (50 µL/well) was added using FlexSation to achieve the final indicated concentrations.

DISCLAIMER

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