Cal-520ER™ AM

Catalog number: 21149
Unit size: 10x50 ug

Component	Storage	Amount (Cat No. 21149)
Cal-520ER™ AM	Freeze (< -15 °C), Minimize light exposure	10x50 ug

OVERVIEW

Cal-520ER™ has been designed to monitor the change of calcium ion (Ca2+) in the endoplasmic reticulum (ER). Cal-520ER™ AM is cell permeable, thus can be readily used in live cells. The importance of calcium signaling in cell health and disease is the major driving force in current research of intracellular calcium homeostasis. Ca2+ release from ER and other calcium stores seems to be the crucial factor in the activation of many cellular functions. Significant changes in ER Ca2+ content and dynamics have been implicated in the activation of the ER stress response, abnormal autophagy, and cell death which leads to a variety of pathological conditions. Cal-520ER is a low-affinity Ca2+ indicator that can be used to record fast Ca2+ signals and to measure the kinetics of Ca2+ currents. Compared to Oregon Green BAPTA-5N and to Fluo4FF, Cal-520ER offers a superior signal-to-noise ratio providing the optimal characteristics for this important type of biophysical measurement. This ability is the result of a relatively high fluorescence at zero Ca2+, necessary to detect enough photons at short exposure windows, and a high dynamic range leading to large fluorescence transients associated with short Ca2+ influx periods.

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

Cal-520ER™ AM Stock Solution

1. Prepare a 2 to 5 mM stock solution of Cal-520ER™ AM in high-quality, anhydrous DMSO.

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

PREPARATION OF WORKING SOLUTION

Cal-520ER™ AM Working Solution

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

2. Prepare a 2 to 20 µM Cal-520ER™ AM working solution in a buffer of your choice (e.g., Hanks and Hepes buffer) with 0.04% Pluronic® F-127, and 1-2 mM of probenecid (final in well concentration will be 0.5-1mM). For most cell lines, Cal-520ER™ AM at a final concentration of 10-20 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-520ER™ AM. A variety of Pluronic® F-127 solutions can be purchased from AAT Bioquest.

   Note: If your cells contain organic anion-transporters, probenecid 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 Cal-520ER™ 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 2 to 3 hours.

   Note: The incubation time for specific cell lines may require optimization to improve signal intensities.

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

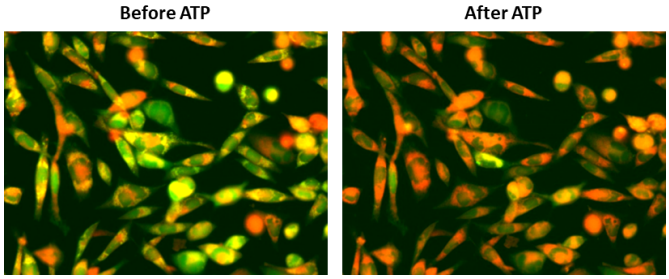

Figure 1. Endogenous P2Y receptor response to ATP in CHO-K1 cells. CHO-K1 cells were seeded overnight at a density of 40,000 cells per 100 µL per well in a 96-well black wall/clear bottom Costar plate. Next, 100 µL of 10 µM Cal-520ER™ AM in HHBS with 1 mM probenecid was added to each well. The cells were then incubated at 37 °C for 3 hours. In the final 30 minutes of incubation, the Cell Navigator® Live Cell Endoplasmic Reticulum (ER) Staining Kit *Red Fluorescence* (Cat# 22636) was added to stain the ER. After incubation, the dye-loading mediums were replaced with 100 µL HHBS containing 1 mM probenecid. The cells were imaged with a fluorescence microscope (Olympus IX71) using the FITC channel, both before and after the addition of 50 µL of 300 µM ATP.

DISCLAIMER

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