Cal-520N™, AM

Catalog number: 21146
Unit size: 10x50 ug

Component	Storage	Amount (Cat No. 21146)
Cal-520N™, AM	Freeze (< -15 °C), Minimize light exposure	10x50 ug

OVERVIEW

Cal-520® 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. Compared to other Cal-520® indicators, Cal-520N™ has the lowest affinity to calcium with Kd ~ 90 uM.

KEY PARAMETERS

Flow cytometer

Emission	530/30 nm filter
Excitation	488 nm laser
Instrument specification(s)	FITC channel

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-520N™ AM Stock Solution

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

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

PREPARATION OF WORKING SOLUTION

Cal-520N™ AM Working Solution

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

2. Prepare a 2 to 20 µM Cal-520N™ 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, Cal-520N™ 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-520N™ 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 Cal-520N™ 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 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.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.