Cal-500™ AM

Catalog number: 20412
Unit size: 10x50 ug

Component	Storage	Amount (Cat No. 20412)
Cal-500™ AM	Freeze (< -15 °C), Minimize light exposure	10x50 ug

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. Cal-500™ is an UV-excitable calcium indicator with maximum emission at ~500 nm. It has a Stokes Shift larger than 100 nm. It can also be well excited with the 405 nm violet laser with a moderate calcium affinity of Kd ~303 nM. In CHO and HEK cells Cal-500™ AM has great cellular calcium response. The excitation spectra of Cal-500 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, FITC/Alexa Fluor® 488 labeled antibodies or other red fluorescent probes.

KEY PARAMETERS

Fluorescence microscope

Emission	DAPI
Excitation	DAPI
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Cutoff	470
Emission	500
Excitation	400
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-500™ AM Stock Solution

1. Prepare a 2 to 5 mM stock solution of Cal-500™ AM in anhydrous DMSO.

   Note: When reconstituted in DMSO, Cal-500™ AM is a clear, colorless solution.

PREPARATION OF WORKING SOLUTION

Cal-500™ AM Working Solution

1. On the day of the experiment, either dissolve Cal-500™ AM in DMSO or thaw an aliquot of the indicator stock solution to room temperature.

2. Prepare a 2 to 20 µM Cal-500™ 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-500™ 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-500™ 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-500™ 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 30 to 60 minutes.

   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 DAPI filter set or a fluorescence plate reader containing a programmable liquid handling system such as an FDSS, FLIPR, or FlexStation, at Ex/Em = 400/500 nm cutoff 470 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

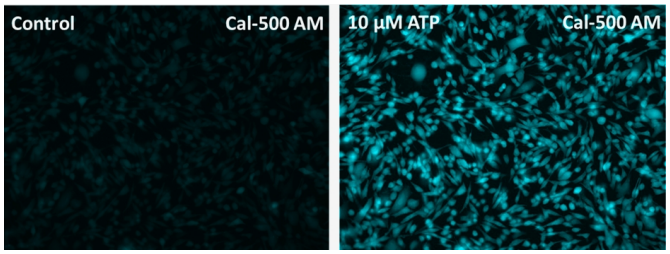

Figure 1. Response of endogenous P2Y receptor to ATP in CHO-K1 cells. CHO-K1 cells were seeded overnight at 40,000 cells per 100 µL per well in a 96-well black wall/clear bottom costar plate. 100 µL of Cal-500™ AM in HHBS with probenecid were added into the wells, and the

cells were incubated at 37 °C for 60 min. The dye-loading medium was replaced with 200 µL HHBS. Images were taken before and after adding 50 µL of 10 µM ATP via a fluorescence microscope (Keyence) using 405 nm and 465 nm long pass filters.

DISCLAIMER

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