Cal-590™ AM

Catalog number: 20510, 20511, 20512
Unit size: 5x50 ug, 10x50 ug, 1 mg

Component	Storage	Amount (Cat No. 20510)	Amount (Cat No. 20511)	Amount (Cat No. 20512)
Cal-590™ AM	Freeze (< -15 °C), Minimize light exposure	5x50 ug	10x50 ug	1 vial (1 mg)

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. Rhod-2 is most commonly used among the red fluorescent calcium indicators. However, Rhod-2 AM is only moderately fluorescent in live cells upon esterase hydrolysis, and has very small cellular calcium responses. Cal-590™ has been developed to improve Rhod-2 cell loading and calcium response while maintaining the spectral wavelength of Rhod-2, making it compatible with TRITC/Cy3® filter set. In CHO and HEK cells Cal-590™ AM has cellular calcium response that is much more sensitive than Rhod-2 AM. The spectra of Cal-590 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 or FITC/Alexa Fluor® 488 labeled antibodies.

KEY PARAMETERS

Fluorescence microscope

Excitation	TRITC/Cy3
Emission	TRITC/Cy3
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Excitation	540
Emission	590
Cutoff	570
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-590™ AM Stock Solution
Prepare a 2 to 5 mM stock solution of Cal-590™ AM in high-quality, anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

Cal-590™ AM Working Solution
On the day of the experiment, either dissolve Cal-590™ 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-590™ 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-590™ 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-590™ 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 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 TRITC/Cy3 filter set or a fluorescence plate reader containing a programmable liquid handling system such as an FDSS, FLIPR, or FlexStation, at Ex/Em = 540/590 nm cutoff 570 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

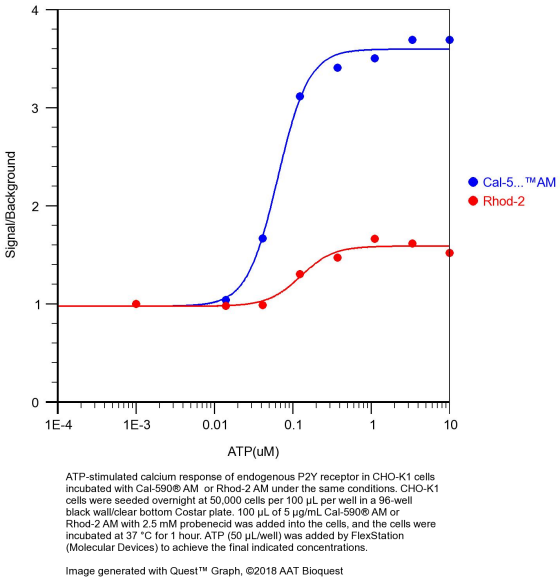

Figure 1. ATP-stimulated calcium response of endogenous P2Y receptor in CHO-K1 cells incubated with Cal-590® AM or Rhod-2 AM under the same conditions. CHO-K1 cells were seeded overnight at 50,000 cells per 100 µL per well in a 96-well black wall/clear bottom Costar plate. 100 µL of 5 µg/mL Cal-590® AM or Rhod-2 AM with 2.5 mM probenecid was added into the cells, and the cells were incubated at 37 °C for 1 hour. ATP (50 µL/well) was added by FlexStation (Molecular Devices) to achieve the final indicated concentrations.

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.