Cal Red™ R525/650 AM

Catalog number: 20590, 20591
Unit size: 1 mg, 10x50 ug

Component	Storage	Amount (Cat No. 20590)	Amount (Cat No. 20591)
Cal Red™ R525/650 AM	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)	10x50 ug

OVERVIEW

The intracellular calcium flux assay is a widely used method for monitoring the activities of GPCRs and calcium channels. To quantify the intracellular calcium concentration, ratiometric fluorescent calcium indicators are preferred because the ratio is directly related to the calcium concentration and independent of the cell numbers and dye loading concentration. However, the most popular ratiometric calcium indicators (such as Fura-2 and Indo-1) have certain limitations such as lower sensitivity, UV excitation, and not compatible with HTS screening filter set. Cal Red™ R525/650 has been developed as a new 488 nm-excitable ratiometric fluorescence calcium indicator. Cal Red™ R525/650 is weakly fluorescent, and once enters cells, the lipophilic AM blocking groups are cleaved by intracellular esterase, resulting in a negatively charged fluorescent dye retained well in cells with excitation close to 488 nm and two emissions at 525 nm and 650 nm. When cells are stimulated with a bioactive compound, the receptor initiates the release of intracellular calcium, which is chelated by Cal Red™ R525/650. The emission signal is increased at 525 nm and decreased at 650 nm when excited at 488 nm. The excitation and emission wavelength of Cal Red™ R525/650 are compatible with common filter sets with minimal damage to cells, making it a robust tool for evaluating and screening GPCR agonists and antagonists as well as calcium channel targets.

KEY PARAMETERS

Fluorescence microscope

Excitation	FITC
Emission	FITC
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Excitation	490
Emission	525, 660
Cutoff	515, 630
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 Red™ R525/650 AM Stock Solution
Prepare a 2 to 5 mM stock solution of Cal Red™ R525/650 AM in high-quality, anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

Cal Red™ R525/650 AM Working Solution
On the day of the experiment, either dissolve Cal Red™ R525/650 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 Red™ R525/650 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 Red™ R525/650 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 Red™ R525/650 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 1 hour 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 a FlexStation, at Ex/Em_1 = 490/525 nm cutoff 515 nm and Ex/Em_2 = 490/660 nm cutoff 630 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

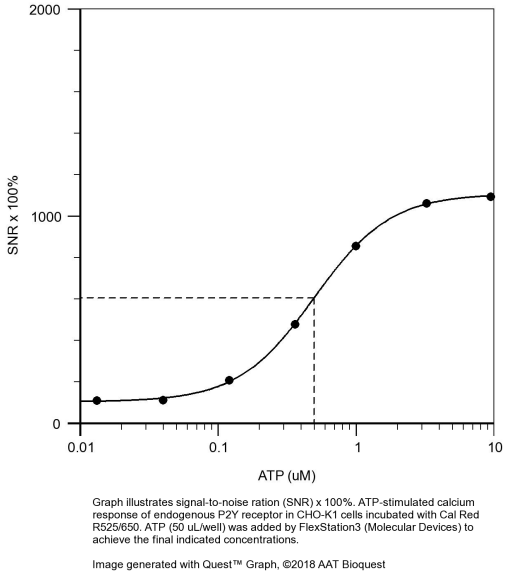

Figure 1. Graph illustrates signal-to-noise ration (SNR) x 100%. ATP-stimulated calcium response of endogenous P2Y receptor in CHO-K1 cells incubated with Cal Red R525/650. ATP (50 uL/well) was added by FlexStation3 (Molecular Devices) to achieve the final indicated concentrations.

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.