Cal Red™ R525/650 potassium salt

Catalog number: 20588
Unit size: 5x50 ug

Component	Storage	Amount (Cat No. 20588)
Cal Red™ R525/650 potassium salt	Freeze (< -15 °C), Minimize light exposure	5x50 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 well excited at 488 nm with two emissions at 525 nm and 650 nm. Upon binding calcium, the emission signal of Cal Red™ R525/650 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.

SAMPLE EXPERIMENTAL PROTOCOL

Sample protocol to determine Kd values

Calcium calibration can be carried out by measuring the fluorescence intensity of the salt form (25 to 50 µM in
fluorescence microplate readers) of the indicators in solutions with precisely known free Ca2+ concentrations. Calibration
solutions can be used based on 30 mM MOPS EGTA Ca2+ buffer. In general, water contains trace amount of calcium ion. It
is highly recommended to use 30 mM MOPS + 100 mM KCl, pH 7.2 as buffer system. One can simply make a 0 and 39 µM
calcium stock solutions as listed below, and these 2 solutions are used to make a serial solution of different Ca2+
concentrations.

A. 0 µM calcium: 30 mM MOPS + 100 mM KCl, pH 7.2 buffer + 10 mM EGTA

B. 39 µM calcium: 30 mM MOPS + 100 mM KCl, pH 7.2 buffer + 10 mM EGTA + 10 mM CaCl2

To determine either the free calcium concentration of a solution or the Kd of a single-wavelength calcium indicator,
the following equation is used:

$$[Ca]free = Kd[F - Fmin]/[Fmax - F]$$

Where F is the fluorescence intensity of the indicator at a specific experimental calcium level, Fmin is the
fluorescence intensity in the absence of calcium and Fmax is the fluorescence intensity of the calciumsaturated probe.

The dissociation constant (Kd) is a measure of the affinity of the probe for calcium. The calcium-binding and
spectroscopic properties of fluorescent indicators vary quite significantly in cellular environments compared to calibration
solutions. In situ response calibrations of intracellular indicators typically yield Kd values significantly higher than in vitro
determinations. In situ calibrations are performed by exposing loaded cells to controlled Ca2+ buffers in the presence of ionophores such as A-23187, 4-bromo A-23187 and ionomycin. Alternatively, cell permeabilization agents such as digitonin
or Triton® X-100 can be used to expose the indicator to the controlled Ca2+ levels of the extracellular medium.

EXAMPLE DATA ANALYSIS AND FIGURES

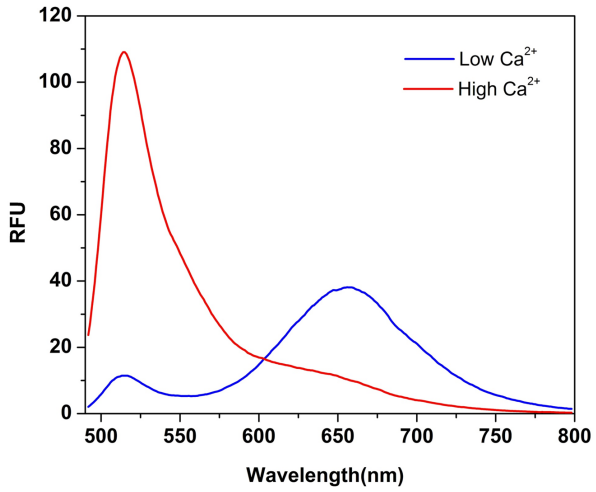

Figure 1. Fluorescence emission spectra of Cal Red™ R525/650 (calcium bound).

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