Cal-670™, potassium salt

Catalog number: 20455
Unit size: 10x50 ug

Component	Storage	Amount
Cal-670™, potassium salt	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-670™ is a near infrared (NIR) calcium indicator with maximum emission at ~675 nm. It can be well excited with the red lasers at 633 nm or 647 nm with a moderate calcium affinity of Kd ~853 nM. Cal-670™ is one of the very few calcium indicators that can be potentially used for in vivo imaging since it has a NIR fluorescence.

SAMPLE EXPERIMENTAL PROTOCOL

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 Ca^{2+} concentrations. Calibration solutions can be used based on 30 mM MOPS EGTA Ca^{2+} 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 Ca^{2+} 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 $CaCl_2$

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

$$[Ca]_{Free} = K_d [F - F_{min}] / [F_{max} - F]$$

Where F is the fluorescence intensity of the indicator at a specific experimental calcium level, F_{min} is the fluorescence intensity in the absence of calcium and F_{max} is the fluorescence intensity of the calciumsaturated probe.

The dissociation constant (K_d) 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 K_d values significantly higher than in vitro determinations. In situ calibrations are performed by exposing loaded cells to controlled Ca^{2+} 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 Ca^{2+} levels of the extracellular medium. The fluorescence can be measured at Ex/Em = 650/675 nm. (Cutoff = 665 nm)

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Ca2+ samples. We recommend using the Online Four Parameter Logistics Calculator which can be found at:

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

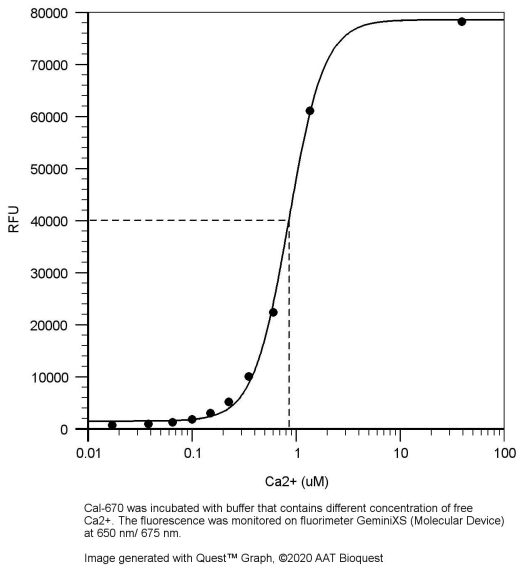

Figure 1. Cal-670 was incubated with buffer that contains different concentration of free Ca2+. The fluorescence was monitored on fluorimeter GeminiXS (Molecular Device) at 650 nm/ 675 nm.

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