Fluorescent Calcium Indicators

Introduction

Calcium flux assays are preferred methods in drug discovery for screening G protein coupled receptors (GPCR). Our Quest Fluo-8™ and Rhod-4™ serial calcium detection reagents are the brightest green and red calcium indicators. Our other calcium indicators such as Fluo-3, Fura-2, Indo-1, Rhod-5N, and Rhod-2 AM also have the highest quality compared to those on the market.

Table 1. Spectral and Ca^{2+}–Binding Properties of Calcium Detection Reagents

Ca^{2+} Indicator	Catalog Numbers		Excitation	Emission	K_d of Ca^{2+}-Binding
	Salt	AM Ester			
Quest Fluo-8™	21088	21080, 21081, 21082, 21083	490 nm	514 nm	389 nM
Quest Fluo-8H™	21095	21090, 21091	490 nm	514 nm	232 nM
Quest Fluo-8L™	21098	21096, 21097	490 nm	514 nm	1.86 uM
Fluo-3	21016 21017 21018	21010, 21011, 21012, 21013, 21014	506 nm	526 nm	325 nM
Fura-2	21025 21026	21020, 21021, 21022, 21023, 21024	340/380 nm	510 nm	140 nM
Indo-1	21040 21044	21030, 21032, 21033, 21036, 21038	355 nm	400/475 nm	230 nM
Quest Rhod-4™	21028	21120, 21121 21122, 21123	530 nm	555 nm	525 nM
Rhod-2	21067 21068	21060, 21062, 21063, 21064, 21065	549 nm	578 nm	570 nM
Rhod-5N	21072	21070	551 nm	577 nm	320 uM

Storage Conditions

Store at –20 ^{0}C, protected from light. Expiration date is 6 months from the date of receipt.

Use of Calcium indicator AM Esters

1. Load Cells with Calcium Indicator AM Esters:

AM esters are the non-polar esters that readily cross live cell membranes, and rapidly hydrolyzed by cellular esterases inside live cells. AM esters are widely used for loading a variety of polar fluorescent probes into live cell non-invasively. However, cautions must be excised when AM esters are used since they are susceptible to hydrolysis, particularly in solution. They should be reconstituted in high-quality, anhydrous dimethylsulfoxide (DMSO) just before use. DMSO stock solutions should be stored desiccated at -20 °C and protected from light. Under these conditions, AM esters should be stable for several months.

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.

a) Prepare a 2 to 5 mM AM esters stock solution in high-quality, anhydrous DMSO.
b) On the day of the experiment, either dissolve calcium indicators solid in DMSO or thaw an aliquot of the indicator stock solutions to room temperature. Prepare a working solution of 1 to 10 µM in the buffer of

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your choice (such as Hanks and Hepes buffer) with 0.02% *Pluronic® F-127*. For most cell lines we recommend the final concentration of calcium indicators be 4-5 uM. The exact concentration of indicators required for cell loading must be determined empirically. To avoid any artifacts caused by overloading and potential dye toxicity, it is recommended to use the minimal probe concentration that can yield sufficient signal strength.
Note: The nonionic detergent Pluronic® F-127 is sometimes used to increase the aqueous solubility of calcium indicator AM esters. A variety of Pluronic® F-127 solutions can be purchased from AAT Bioquest.

c) If your cells containing the organic anion-transports, probenecid (1–2.5 mM) or sulfinpyrazone (0.1–0.25 mM) may be added to the cell medium to reduce the leakage of the de-esterified indicators.
Incubate cells with the calcium indicators esters at room temperature or 37 °C for 20 minutes to one hour.
Note: Decreasing the loading temperature might reduce the compartmentalization of the indictor.

d) Wash cells 1-2 times in HHBS or buffer of your choice (containing an anion transporter inhibitor, such as 2.5 mM probenecid, if applicable) to remove excess probes.

e) Run the experiments at desired Ex/Em wavelengths (see Table 1).

2. Measure Intracellular Calcium Responses:

To determine either the free calcium concentration of a solution or the K_d 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 of the indicator at experimental calcium levels, F_{min} is the fluorescence in the absence of calcium and F_{max} is the fluorescence of the calcium-saturated probe. The dissociation constant (K_d) is a measure of the affinity of the probe for calcium. The Ca^{2+}-binding and spectroscopic properties of fluorescent indicators vary quite significantly in cellular environments compared to calibration solutions. *In situ* 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 K_d values of some calcium reagents are listed in Table 1 for your reference.

References

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