Fluorescent Calcium Indicators

I. Introduction

Calcium flux assays are preferred methods in drug discovery for screening G protein coupled receptors (GPCR). Our Fluo-8® and Rhod-4™ serial calcium detection reagents are the brightest green and red calcium indicators while our Cal-520® and Cal-590™ give the highest signal/background ratio for intracellular calcium detection due to their excellent retention in live cells. AAT Bioquest offers other calcium indicators such as Fluo-4, Fluo-3, Fura-2, Indo-1, Rhod-5N, and Rhod-2 AM in the highest possible quality.

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			
Cal-520®	21135, 21136, 21140, 21141	21130, 21131	492 nm	514 nm	320 nM
Cal-520FF™	21144	21142, 21143	492 nm	514 nm	9.2 µM
Cal-590™	20515, 20518	20510, 20511, 20512	573 nm	588 nm	561 nM
Cal-630™	20535, 20538	20530, 20531 20532	608 nm	626 nm	792 nM
Cal Green™ 1*	20500	20501, 20502	506 nm	531 nm	190 nM
Cal Red™ R525/650	20588	20590, 20591	492 nm	525/650 nm	330 nM
Fluo-8®	21086, 21087, 21088, 21089	21080, 21081 21082, 21083	490 nm	514 nm	389 nM
Fluo-8FF™	21102, 21103	21104, 21105	490 nm	514 nm	10 µM
Fluo-8H™	21095	21090, 21091	490 nm	514 nm	232 nM
Fluo-8L™	21098, 21099, 21100, 21101	21096, 21097	490 nm	514 nm	1.86 µM
Fluo-4	20555, 20556	20550, 20551 20552	494 nm	516 nm	345 nM
Fluo-3	21016, 21017 21018	21010, 21011 21012, 21013	506 nm	526 nm	325 nM
Fluo-3FF	21019	21014	506 nm	526 nm	10 µM
Fura-2	21025, 21026	21020, 21021 21022, 21023	340/380 nm	510 nm	140 nM
Fura FF	21028	21027	340/380 nm	510 nm	5.5 µM
Fura-8	21057, 21058	21055, 21056	354/415 nm	524 nm	260 nM
Fura-8 FF	20621	20620	354/415 nm	524 nm	6 µM
Fura Red	21045, 21047	21046, 21048	436/471 nm	630/652	400 nM
Indo-1	21040, 21044	21030, 21032 21033, 21036	355 nm	400/475 nm	230 nM
Rhod-4™	21128, 21119 21128, 21129	21120, 21121 21122, 21123	530 nm	555 nm	525 nM
Rhod-2	21067, 21068	21060, 21062 21063, 21064	549 nm	578 nm	570 nM
Rhod-FF	21075, 21076	21077, 21078	549 nm	549 nm	19 µM
Rhod-5N	21072	21070	551 nm	577 nm	320 uM

* Cal Green™ 1 is the same molecule to Calcium Green-1

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Table 2. Dextran, Biotin or Biocytin conjugated Fluorescent Calcium Indicators

Cat. #	Product Name	Unit	MW	Ex (nm)[2]	Em (nm)[2]
20605	Cal-520® -Biotin Conjugate	5x50 µg	1112.40	492	514
20606	Cal-520® -Biocytin Conjugate	5x50 µg	1341.55	492	514
20600	Cal-520®-Dextran Conjugate *MW 3,000*	1 mg	~4,000	492	514
20601	Cal-520®-Dextran Conjugate *MW 10,000*	5 mg	~11,000	492	514
20508	Cal-590™-Dextran Conjugate *MW 3,000*	1 mg	~4,000	573	588
20509	Cal-590™-Dextran Conjugate *MW 10,000*	1 mg	~11,000	573	588
20545	Cal-630™-Dextran Conjugate *MW 3,000*	1 mg	~4,000	608	626
20546	Cal-630™-Dextran Conjugate *MW 10,000*	1 mg	~11,000	608	626

II. Storage Conditions

Store at –20 ^{0}C, protected from light. Expiration date is 12 months from the date of receipt.

III. 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). 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 2 to 20 µM in the buffer of your choice (such as Hanks and Hepes buffer) with 0.04% *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 (such as CHO cells) containing the organic anion-transports, probenecid (2–5 mM) or sulfinpyrazone (0.2–0.5 mM) may be added to the the dye working solution (final in well concentration will be 1-2.5 mM for probenecid, or 0.1 -0.25 mM for sulfinpyrazone) to reduce the leakage of the de-esterified indicators.
Note: A variety of ReadiUse™ probenecid including water soluble sodium salt and stabilized solution can be purchased from AAT Bioquest

d) Add equal volume of the dye working solution (from Step b or c) into your cell plate.

e) Incubate the dye-loading plate room at temperature or 37 °C for 20 minutes (especially Fluo-8 AM) to 2 hours, and then incubate the plate at room temperature for another 30 minutes.
Note1: Decreasing the loading temperature might reduce the compartmentalization of the indictor.
Note2: Incubate the Cal-520 AM longer than 2 hours gives better signal intensity for some cell lines.

f) 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 excess probes.

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

2. Measure Intracellular Calcium Responses:

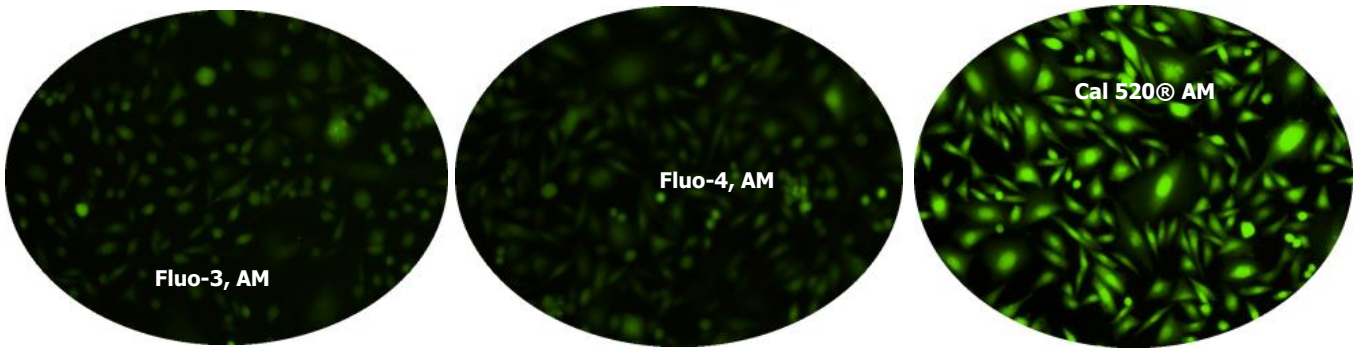

Figure 1. **Response of endogenous P2Y receptor to ATP in CHO-M1 cells without probenecid.** CHO-M1 cells were seeded overnight at 40,000 cells per 100 µL per well in a 96-well black wall/clear bottom costar plate. 100 µl of 4 µM Fluo-3 AM, Fluo-4 AM or Cal 520® AM in HHBS were added into the wells, and the cells were incubated at 37 °C for 2 hour. The dye loading medium were replaced with 100 µl HHBS, 50 µl of 300 µM ATP were added, and then imaged with a fluorescence microscope (Olympus IX71) using FITC channel.

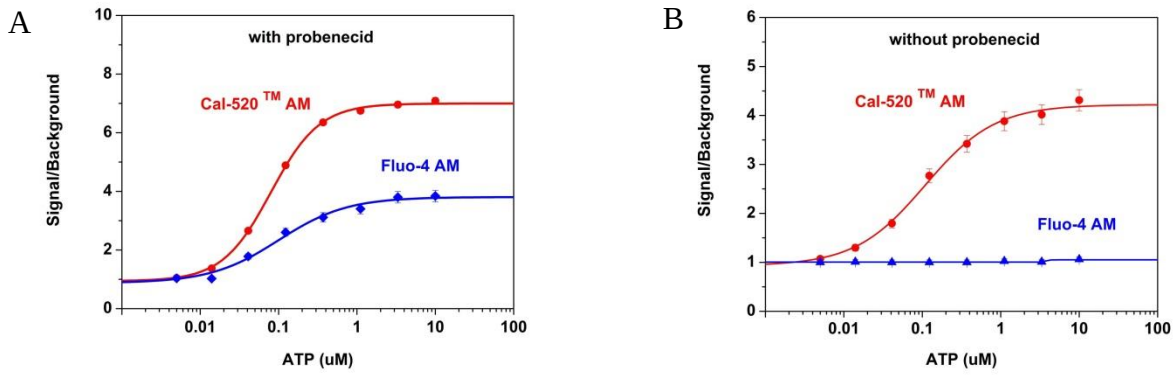

Figure 2. **ATP-stimulated calcium response of endogenous P2Y receptor in CHO-K1 cells measured with Cal-520® or Fluo-4 AM.** CHO-K1cells were seeded overnight in 50,000 cells per 100 µL per well in a 96-well black wall/clear bottom costar plate. 100 µL of 5 µM Fluo-4 AM or the Cal-520® AM with (A) or without (B) 2.5 mM probenecid was added into the cells, and the cells were incubated at 37°C for 2 hours. ATP (50µL/well) was added by FlexStation (Molecular Devices) to achieve the final indicated concentrations.

IV. Use of Calcium indicator Salts

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.

V Use of Calcium indicator Conjugates

Compared to the free ion indicator, dextran conjugates of these same indicators exhibit both reduced compartmentalization and much lower rates of dye leakage. Since the molecular weight of the dextran, net charge, degree of labeling, and nature of the dye may affect the experiment, researchers are advised to consult the primary literature for information specific to the application of interest.

VII. References

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