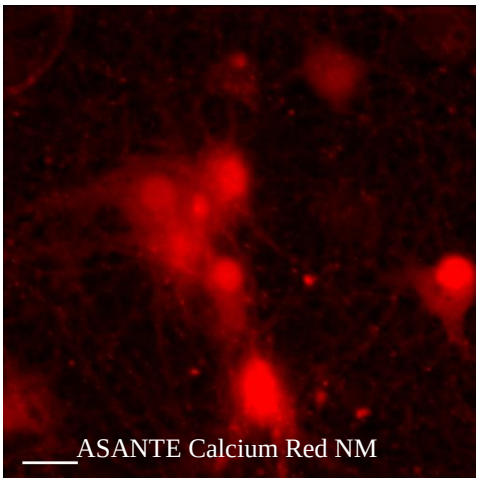
Interchim Innovations

Interbiotech - BioScience Innovations

N03E

New generation of Near Membrane Calcium probes

- **Hydrophobic tail**
  designed for specific distribution in membranes
- Efficient Loading of the dye in the cells
- ASANTE(ACR NM dye) for red Calcium measures
  Excited by 488nm laser.
  Combines long emission wavelengths and ratioing capabilities with very dynamic responses to changing [Ca2+]i.
  Can be Multiplexed with GFP and YFP
- Fura-2 and Indo-1 analogs also available
- Fluo-2 NearMembrane available for non ratiometry



	MW	Absorb.(nm)	Excit.(nm)	Emission(nm)	Kd(nM)	Soluble in		
Fura-2 NM –AM (FIP18)						**FP-AM606A, 1mg**	**FP-AM606B, 20x50µg**	
	1297	364nm	364[b]	502-4905[b]	400nM[b]	DMSO		
Indo-1 NM – AM (FFP18)						**FP-AM608A, 500µg**	**FP-AM608B, 20x50µg**	
	1303	340nm	346[b]	408-475[b]	450nM[b]	DMSO		
ASANTE Calcium Red – AM (ACR NM)						**GV0030, 500µg**	**GV0031, 10x50µg**	
	1300	572nm	488-540[b]	525-650[b]	to be determ.	DMSO		
Fluo-2 NM – AM						**GV0050, 1mg**	**GV0051, 20x50µg**	
	1355	455nm	490[b]	515[b]	to be determined[b]	DMSO		

Price and technical sheet on line

[b]: value for the hydrolysed form, high-low Ca2+ concentration. Not applicable for the AM form.
FFP18 is available as the water soluble K salt form (FP-AM605A).
FIP18 is available as the water soluble K salt form (FP-AM609A).
ASANTE is available as a standard version #FJ297 (KD=400nM), a less affine one (#FJ735 KD=600nM) and a water soluble K salt (#GV004). A green version also exists for sodium detection (ASANTE Natrium Green (ANC) A/E:488-517/540nm) (#FO867).

	Ratiometric mode			Non-ratiometric mode		
	Asante Calcium Red	Fura-2	Indo-1	Fluo	Rhod	**Asante Calcium Red**
Excitation	Argon 488 nm	340/380 nm UV	346 nm UV	Argon 488 nm	550 nm	540 nm
Emission	525 nm/650nm (Red)	505 nm (Green)	405/475 nm	500-550 nm (Green)	578 nm	650 nm (Red)
Ratiometry	Dual emission ratio	Dual excitation ratio	Dual emission ratio	non-ratiometric		enhancement
Stokes Shift	large	large	large	small	small	large
Photobleaching	normal	normal	high	normal	normal	normal
loading	easy	easy	easy	easy	difficult	easy

More about Calcium indicators | Asante Calcium indicator details | Other calcium indicators
(classic, Ratiometric, NearMembrane, Leakage resistant) | Red (ACR) standard, Red NearMembrane, Red LeakRes, Green(ACG) |

More about Calcium indicators

Introduction to classical Near Membrane Calcium probes

Measurement of intracellular free calcium concentration ([Ca2+]i) is typically performed using fluorescent indicators. Ratiometric indicators (such as fura-2) allow calibrated [Ca2+]i measurement by comparison of two excitation or emission wavelengths. However, most ratiometric indicators require excitation with ultraviolet light, their emission wavelengths overlap with those of common fluorescent proteins such as GFP and YFP, and they are generally not suitable for use with standard confocal microscopes. In contrast, calcium indicators with visible excitation wavelengths (such as calcium-green) do not allow the accuracy of ratiometric measurements. The newly designed Asante Calcium Red (ACR) calcium fluorescent indicator, features visible excitation with long excitation and emission wavelength, and ratiometric capabilities.

Ratiometric Calcium probes (NearMem, NM)

Until now, only two useful ratiometric Ca2+ indicators are known: Fura-2 and Indo-1. Fura -2 allows excitation ratiometry, while Indo-1 is principally used for emission ratiometry. Both these indicators require cell-damaging UV excitation expensive UV-transmitting optics.

Near Membrane Calcium probes (NearMem, NM)

Unlike other hydrophobic dyes, our near membrane dyes have a collar to latch the dye into the membrane, leaving the chelating portion to measure calcium in the gates. Our near-membrane UV-excitable indicators **Fura-2 NearMem** (Figure. 6.9, formerly FFP-18) , and **Indo-1 NearMem** (Figure. 6.10, formerly FIP-18), have enjoyed considerable success for their applications, because unlike other hydrophobic indicators, they do not get lost in the membrane.

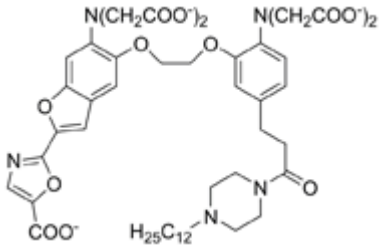


Figure 6.9 Fura-2 NearMem (FFP-18)

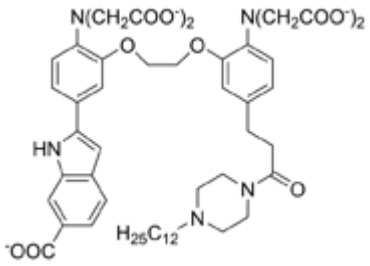


Figure 6.10 Indo-1 NearMem (FIP-18)

Near membrane versions of Asante Calcium Red, Asante Calcium Green and Fluo-2 share the same features, preventing such a phenomenon of near membrane Ca depletion.
We offer the cell-impermeable potassium salts and the cell-permeable AM form of the near membrane dyes.

The application in neutrophils is a classic example of near-membrane study.

The first near-membrane fluorescent calcium indicators were based on Fura-2. The near membrane phenomenon was classically illustrated by the distribution of the dye in the plasma membrane and its response to calcium.
The near membrane versions of Calcium probes such as Indo1 or Fluo3 have a special lipophilic anchor that tethers the dye to the lipid membrane, so that it can report on changes in [ca2+] occurring near the membrane. Unfortunately, the high concentrations of calcium influx quickly saturate current near-membrane versions. Design of low affinity near membrane dyes is ongoing.

Figure 6.11A shows the distribution of the dye Fura-2 NearMem in the plasma membrane.
Figure 6.11B is a plot of fluoresence intensity due to Fura-2 NearMem as a function of the distance from the cell membrane.

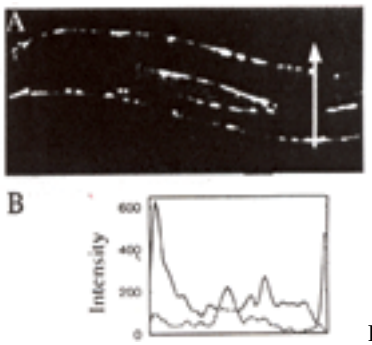


Figure 6.11

Figure 6.12 is a plot of the excitation spectra of a titration of Fura-2 NearMem with calcium, where emission was set at 500 nm.

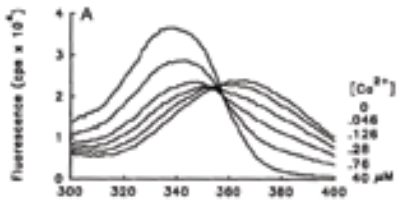


Figure 6.12 :
Fura-2 Near membraneFura-2 near membrane titration

Leakage resistant Calcium probes (LeakRes, LR)

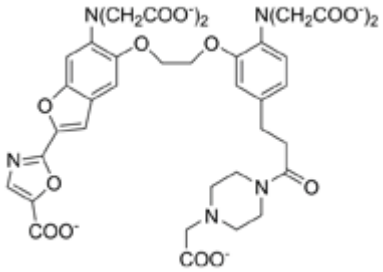
Leakage resistance (LeakRes) versions have a special appendage that increases the intracellular retention – well in excess of an hour.

The first Leakage Resistant Ca2+ indicators, **Fura-2 LeakRes** (Figure 6.5, formerly Fura-PE3) and **Indo-1 LeakRes** (Figure 6.6, formerly Indo-PE3) were developed by Minta and Poenie in the early 1990s. Several literature reference their use. Compared to the corresponding non-LeakRes versions, the LeakRes indicators are identical in fluorescence properties but are retained in the cell for hours. In addition to Fura and Indo, TEFLabs has applied this unique technology to our new indicators Asante Calcium Red and Fluo-2. The LeakRes indicators are available as the K+ salt and the AM forms. (**Asante Calcium Green** has a very high leakage resistance in its base form.)
Under development: dextran versions of our calcium indicators. At this time, in lieu of dextran, we offer leakage resistant versions for injection in cell-impermeable salt forms. In particular, we offer our ultra-resistant Asante Calcium Green.

	Catalog Number	MW (g/mol)	Absorbance (nm)	Excitation (nm)	Emission (nm)	K_d* (nM)	Solubility
Fura-2 LeakRes (K^+ salt)	0110	832	354	335/362 145 (high/low Ca^{2+})	505	145 (224 at 37°C)	H_2O
Fura-2 LeakRes (AM)	0108	1258	371[b]	N/A[b]	N/A[b]	N/A[b]	DMSO
Indo-1 LeakRes (AM)	0144+	1266	346	N/A[b]	N/A[b]	N/A[b]	DMSO
Indo-1 LeakRes (K^+ salt)	0146	1062	346	346	408/475 (high/low Ca^{2+})	260	H_2O
Asante Calcium Red LeakRes (AM)	3150+	1400	472	N/A[b]	N/A[b]	N/A[b]	DMSO
Asante Calcium Red LeakRes (K^+ salt)	3170	1200	555	488 or 540[c]	525/650[c] or 650	to be determined	H_2O
Asante Calcium Green (K^+ salt)	3704	1100	518	488 to 517[d]	540	135[c]	H_2O
Asante Calcium Green (AM)	3700+	1300	469	N/A[b]	N/A[b]	N/A[b]	DMSO
Fluo-2 LeakRes (AM)	0230+	1317	455	N/A[b]	N/A[b]	N/A[b]	DMSO
Fluo-2 LeakRes (K^+ salt)	0234	1113	490	490	515	to be determined	H_2O

a The dissociation constant (Kd) is sensitive to pH, temperature, viscosity, ionic strength, competing ions, and cellular interactions. This Kd was measured in simple aqueous buffers as a guideline to the scientist, who should then calibrate the indicator the cells under study.

b Once the AM ester form permeates the cell membrane, intracellular non-specific esterases hydrolyze the AM ester to yield the indicator in its Ca2+ sensitive salt form.



6.5 Fura-2 LeakRes (Fura-PE3)

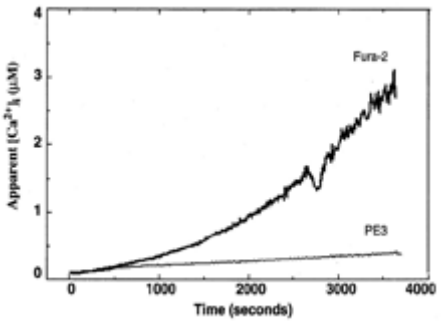
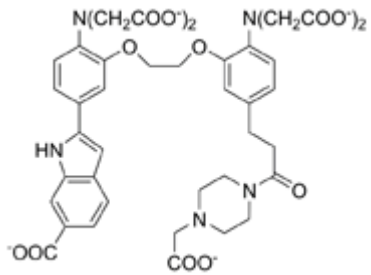


Figure 6.7 Retention of Fura-2 LeakRes and leakage of Fura-2: 322 T lymphoma cells wer loaded with either Fura-2 or Fura-2 LeakRes and set in calcium buffer. Leakage of Fura-2 or Fura-2 LeakRes into the exterior calcium buffer resulted in increased fluorescence overall. This fluorescence was plotted over time.



6.6 Indo-1 LeakRes (Indo-PE3)

Fluo-2 LeakRes, also sold under the name Fluo-8 LeakRes, maintains the characteristics of Fluo-2 /Fluo-8 with additional appendages that allow the indicator to be retained in the cell for hours

Products description

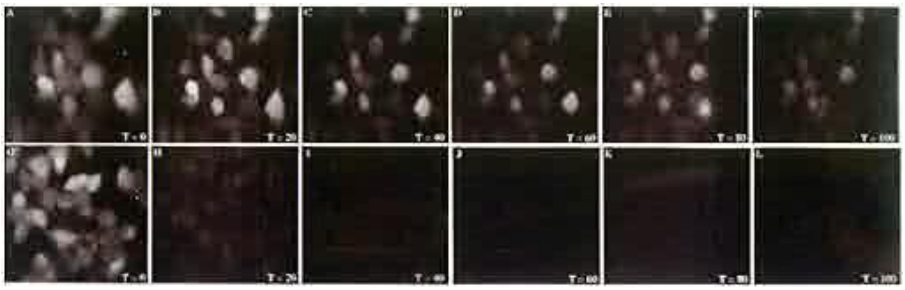
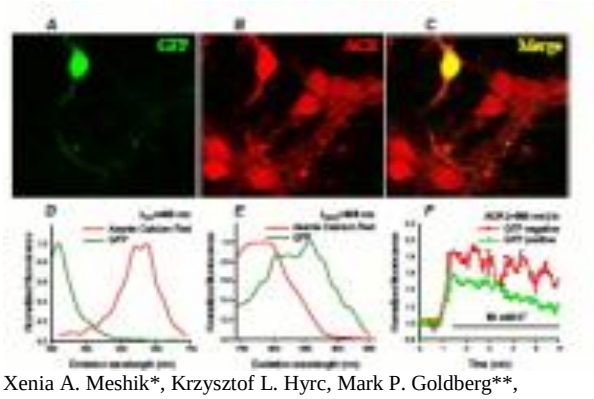


Figure 6.8 shows the slower decrease in fluorescence due to leakage of indicator. The top row shows images of BPV cells loaded with Fura-2 LeakRes, whereas the bottom row shows BPV cells loaded with Fura-2. Images were taken at every 20 minutes. Fura-2 cells show loss of significant fluorescence by T = 40 minutes. Fura-2 LeakRes cells retain fluorescence even at T = 100 minutes.

Figure 6.8 methods: BPV cells, adhered to coverslips, were loaded with Fura-2 LeakRes(AM) or Fura-2(AM) as described in Materials and Methods. Cells were mounted in a Sykes-Moore chamber and placed on a water-jacketed holder of a Zeiss IM-35 microscope. The temperature was maintained at 37oC in the sample chamber by a thermostatically controlled circulating water bath. Images were acquired with a Hammamatsu SIT camera and a Photon Technology Image Master illumination and acquisition system. Images of the same microscope field were recorded at 360 nm excitation at 20-min intervals beginning immediately after cells were washed. Camera gain and intensifier voltages were set based on the brightness of cells at the first time point and maintained constant thereafter. Between the acquisition of light was blocked by a shutter. (A-F) The upper series of photographs shows the pattern of fluorescence change for Fura-2 LeakRes loaded BPV cells. The lower series of photographs (G-L) shows the corresponding changes in Fura-2 loaded BPV cells

Asante Calcium Red (ACR) - standard version

ASANTE (ACR dye) can be excited with wavelengths ranging from 450 to 545 nm, with maximum emission at 640 nm, which allows imaging using the Argon laser available on standard confocal microscopes. The large Stokes' shift allows using it as a single wavelength when excited with 453, 488 or 543 nm laser lines or as a ratiometric indicator by dividing the ACR emission at 640 and 530 nm when using 488 nm excitation. In either case, the indicator offers a large dynamic range as its emission at 640 nm increases about 30 times upon Ca2+ binding, whereas the 530 nm emission is unaffected by Ca2+ and isosbestic. Alternatively, the indicator might be imaged using 750-800 nm two-photon excitation in either single wavelength or ratiometric mode. The long emission wavelength permits [Ca2+]i determination in the presence of YFP or GFP. The dissociation constants for calcium were found to be 400 nM in the absence and 600 nM in the presence of 1 mM Mg2+. ACR fluorescence is sensitive to zinc (Kd=0.6 nM) and magnesium (Kd~20 mM).



Xenia A. Meshik*, Krzysztof L. Hyrc, Mark P. Goldberg**, Washington University School of Medicine, Dept. Neurology, The Hope Center for Neurological Disorders, Alafi Neuroimaging Laboratory, Saint Louis, MO 63110.

ACR can easily be loaded in cells using a standard AM ester loading protocol (5 µM ACR/AM, 1 hour incubation) .

In summary, ***ASANTE (ACR dye) combines long emission wavelengths and ratioing capabilities with very dynamic responses to changing [Ca2+]i.***

Ordering information:

Asante Calcium Red AM ester	FJ2970, 500µg	FJ2971, 10x50µg	FJ297E, 2x50µg
Asante Calcium Red K+ salt	FJ2980, 250µg		FJ298E, 2X25µg

ACR is also available in a Low Affinity (Asante Calcium Red LowAff) version and Near Membrane(Asante Calcium Red NearMem) and Leakage Resistant (Asante Calcium Red LeakRes) versions are currently under development

Datas

Excitation: 488 nm (ratiometric mode)
or 540 nm (non-ratiometric mode)
Emission: 525/650 nm (ratiometric mode)
or 650 nm (non-ratiometric mode)
kd: 400 nM
Solubility: H20 (salt form) DMSO (AM form)
Molecular Weight: 1000g/mol (salt) 1200 g/mol (AM)

Typical loading protocol for AM ester of ACR :

(1) Prepare stock solution of 1 – 10 mM ACR (AM) in DMSO

(2) Incubate cells in 3 – 10 µM ACR (AM) in aqueous medium containing ~0.02% Pluronic F-127 (premix the requisite volume of ACR (AM) stock in DMSO with the desired volume of 15% wt/vol stock of Pluronic in DMSO before dispersal into aqueous medium)

(3) Incubate 45 – 60 minutes at room temperature

(4)Wash cells and maintain cells in fresh medium for ~40 minutes at room temperature to ensure complete intracellular hydrolysis of the AM ester (J Kao, University of Maryland)

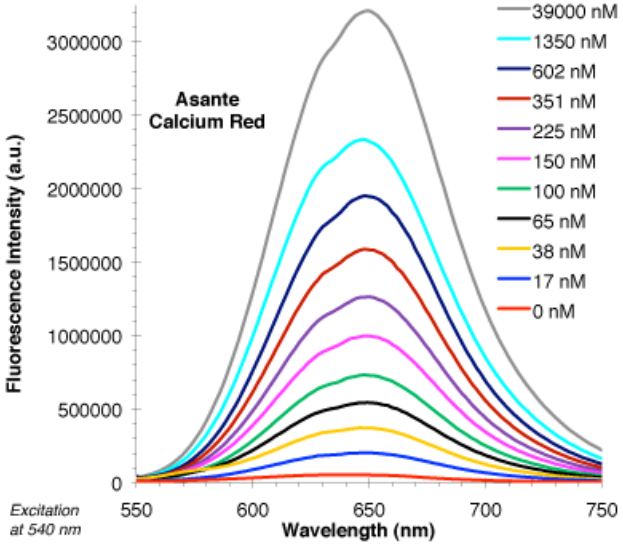
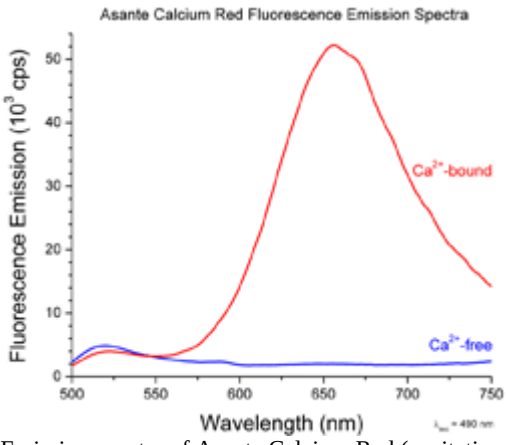


FIG. 4.1 Fluorescence emission traces of a titration of Asante Calcium Red (excitation at 540 nm) showing a calcium-dependent enhancement of fluorescence but not a shift of wavelength.

Asante Calcium Red ratiometric titration:



Emission spectra of Asante Calcium Red (excitation at 488 nm) acquired at zero (Ca2+-free) and saturating (Ca2+-bound) concentrations of Ca2+. The spectral change can be used for emission ratiometry. [J. Kao, Univ of Maryland]

Asante Calcium Red Low Affinity (ACR lowAff)

Asante Calcium Red Low Affinity enables studies where large concentrations of calcium are expected. ACR lowAff emits fluorescence at long wavelengths, where there is virtually no interference from cellular autofluorescence.

Low affinity (lowAff, formerly designated as "FF") versions enable studies where a large concentration of calcium is expected. They have two major advantages over their high affinity counterparts. They cause reduced buffering of intracellular calcium and reduced perturbation of calcium transients, and they allow for measurements of shorter-lived transients

Datas
Excitation: 488 nm (ratiometric mode) or 540 nm (non-ratiometric mode)
Emission: 525/650 nm (ratiometric mode) or 650 nm (non-ratiometric mode)
kd: 6.65 nM
Solubility: H20 (salt form) DMSO (AM form)
Molecular Weight: 1000g/mol (salt) 1200 g/mol (AM)

Ordering information:

Asante Calcium Red FF, AM ester	FJ3750, 500µg	FJ3751, 10x50µgg
Asante Calcium Red FF, K+ salt	FJ3770, 250µg	

Asante Calcium Red NearIR (ACR NearIR)

Until now, there have been no Ca2+ indicators that emit in the near-IR wavelength range. The newly designed Asante Calcium NearIR (ACnIR) is a non-ratiometric Ca2+ indicator with excitation maximum at 630 nm and fluorescence emission peaking at 690 nm. A fluorescence emission titration (Figure 4.6) shows indicator response to Ca2+. Cellular experiments is available on inquire.

Datas
Excitation: 635 nm
Emission: 590 nm
kd: - nM
Solubility: H20 (salt form) DMSO (AM form)
Molecular Weight: - g/mol (salt) - g/mol (AM)

Ordering information:

Asante Calcium Red FF, AM ester	FJ3750, 500µg	FJ3751, 10x50µg
Asante Calcium Red FF, K+ salt	FJ3770, 250µg	

Asante Calcium Red Leakage resistant

Please inquire.
* Non décrit ici car pas dispo/web TEF

Asante Calcium Green (ACG)

ACG's high fluorescence quantum efficiency makes it the brightest Ca2+ indicator with the highest fluorescent dynamic range to date. This exceptional brightness and dynamic range allows ACG to report both small and large Ca2+ signals with good signal-to-noise.TEFLabs plans to offer multiple versions of ACG with a range of Kds. Improved methods of non-invasive cell loading are being developed.

In the past, the nonratiometric Fluo dyes have been very useful and popular because they have a large dynamic range (Fmax/Fmin ≈ 100). Asante Calcium Green (ACG) is much brighter than the Fluo dyes with an even larger dynamic range: Fmax/Fmin = 220 (Figure 4.7), where the relevant quantum efficiencies are: Ca2+-free Q = 0.00225, Ca2+-bound Q = 0.495. Figure 4.8 compares the quantum efficiency of Ca2+-bound ACG to fluorescein. (on inquire)

Ordering information:

Asante Calcium Green, AM ester	1B0280, 500µg
Asante Calcium Green, K+ salt	1B0290, 250µg

Datas

Excitation: 517 nm Emission: 540 nm kd: 135 nM
Solubility: H20 (salt form) DMSO (AM form)
Molecular Weight: 1200 g/mol (salt) - 1400 g/mol (AM)

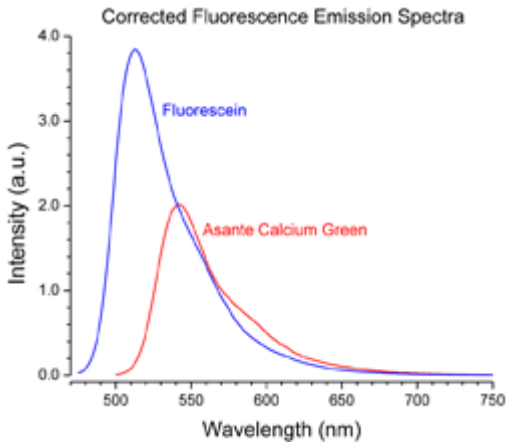


Figure. 4.8 ACG (Ca2+-bound form) quantum efficiency relative to fluorescein

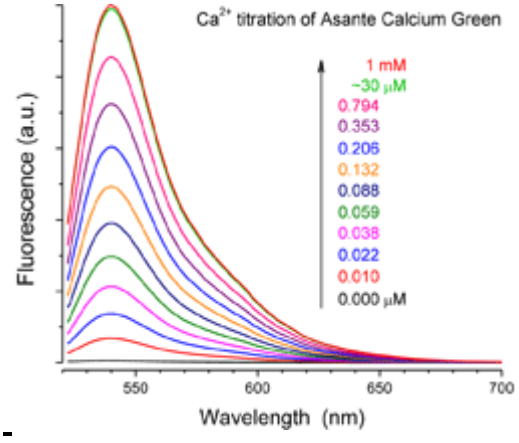


Figure. 4.7 ACG titration

ACG's high fluorescence quantum efficiency makes it the brightest Ca2+ indicator to date. Its exceptional brightness and dynamic range allows ACG to report both small and large Ca2+ signals with good signal-to-noise. Figure 4.9 shows ACR responses to neuronal Ca2+ transients evoked by depolarizations ranging in duration from 1 to 1000 msec. ACG (K+ salt) in rat vagal sensory neurons in whole-cell patch configuration. Calcium signals evoked by depolarization in rat vagal sensory (nodose ganglion) neurons. 50 µM Asante Ca Green K+ salt was included in the intracellular solution filling a patch electrode. Whole-cell configuration was established to allow loading of the indicator from the patch electrode into the cytosol of the neuron. The holding potential of the neuron was Vm = −70 mV. The neuron received a series of step depolarizations from −70 to +10 mV that ranged in duration from 1 − 1,000 msec. Intracellular Ca2+ signals evoked by the depolarizing steps were recorded as increases in indicator fluorescence. For ease of quantitative comparison, fluorescence signals are reported as fractional change of fluorescence relative to baseline fluorescence intensity (ΔF/F0). (J Kao, University of Maryland)

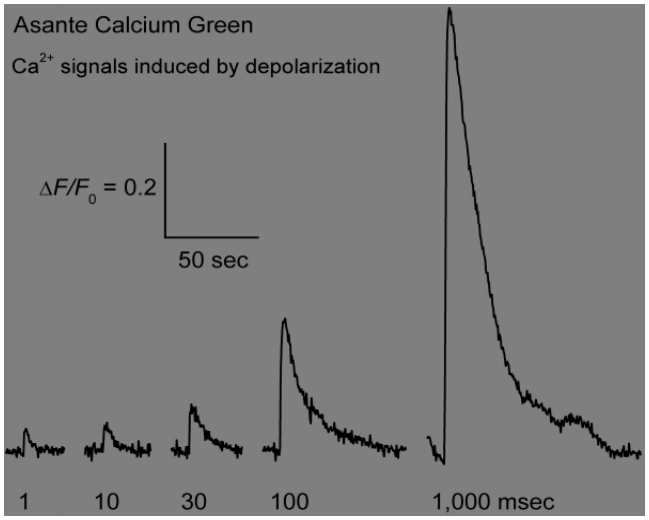


Figure 4.9: ACG responses to neuronal Ca2+ transients evoked by depolarizations ranging in duration from 1 to 1000 msec. ACG was introduced into rat vagal sensory (nodose ganglion) neurons through a whole-cell patch electrode (electrode filling solution containing 50 µM ACG K+salt). The holding potential of the neuron was Vm = −70 mV. The neuron received a series of step depolarizations from −70 to +10 mV that ranged in duration from 1 − 1000 msec. Intracellular Ca2+ signals evoked by the depolarizing steps were recorded as increases in indicator fluorescence. For ease of quantitative comparison, fluorescence signals are reported as fractional change of fluorescence relative to baseline fluorescence intensity (ΔF/F0). [J Kao, Univ. of Maryland Medical School]

Under development: Multiple versions of ACG with a range of Kds.
Improved methods of non-invasive cell loading

Fura2 LeakRes (Fura PE3) #AM603A & AM604A

Please inquire./cat LS2006

Fura2 LeakRes, AM ester AM603A, 1mg AM603C, 20x50µg
Fura2 LeakRes, 6K+ salt AM604A, 500µg

Indo1 LeakRes (Indo PE3) #AM602A & AM601A

Please inquire./cat LS2006

Indo1 LeakRes, AM ester AM602A, 1mg AM602B, 10x50µg
Indo1 LeakRes, 6K+ salt AM601A, 500µg

Fluo2 LeakRes (Fluo-8) #CP750

Please inquire / see Fluo8/cat LS2006, PHBExxx

* Fluo2 LeakRes =semble Fluo 8 !!: a vérifier/+pblm MW donné/tef (877 g/mol (salt) 1047 g/mol (AM)) different du tableau!!!
refTEF.0230 à 0238 non crées sur MAI…

Related products

3D culture supports for cell screening assays, µconfocal imaging, IHC/IF, and angiogenesis exp.: **Cosigel**, **Cosimatric**, and more	BE007a
Cell Viability Assays: **UptiBlue**, **CCK8**, MTT...	BE044b
PMA: selective Detection of Viable Bacteria Using PMA Dye in Conjunction with qPCR	SHBTMp
Endotoxin Detection and removal (LAL assay, EndoLISA | DetoxiGel, EndoTrap)	BE024e
NucView: Caspase detection in living cells	SHBTMn
Fluo8 calcium assay: 24 fold more sensitive than Fluo3, and better for kinetics (photostable)	BE044b
SOD Assay: higher performance using the WST method (water soluble formazan)	FT-S07410
Nuclear Receptor Assays – **Indigo** PPARα,δ,γ, LXRα,β, ERα, FXR, MR assays	BE092i
Cytoxilux & Grantoxilux apoptosis / cytotoxicity assay – caspase mediated – granzyme B mediated	BE155n
Cell Culture suppliers **Cell BioAssays suppliers**	SH000c

Information inquire

Reply by Fax : +33 (0) 4 70 03 82 60 or email at interbiotech@interchim.com

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