Asante Calcium Red - Next Generation Calcium Indicator

One indicator, two excitation-dependent emission response modes

emission ratiometric mode (Figure 1)

- Single excitation at argon laser 488 nm, dual emission at 520 nm and 650 nm
- Greater than twenty fold emission enhancement at 650 nm and decrease to about half of emission at 520 nm, upon saturating with calcium
- Ratiometry eliminates variables such as dye concentration, optical path, and instrumental variations to give an accurate and calibratable reading.
- NOT hydrophobic like Fura-Red
- FULLY longwave visible, unlike Fura-Red and BTC
- NOT a dual fluorophore, artificially ratiometric system, such as Fluo with SNARF

non-ratiometric mode (Figures 2 and 3)

- Excitation 540 nm, calcium-dependent emission enhancement at 650 nm (Stokes shift 110 nm)
- Greater than fifty fold emission enhancement upon saturating with calcium
- Longer wavelength eliminates cellular autofluorescence encountered with Fluo indicators.
- Virtually nonfluorescent in the absence of calcium, unlike the longer wavelength Rhod dyes

Asante Calcium Red Properties

- K_d of ~300 to 400 nM
- pK_a of ~5.5
- More than twice as bright as Fura-Red; not as bright as Fluo dyes, however
- AM ester is nonfluorescent
- Potassium Salt product specifications
- AM ester product specifications
- Potassium Salt MSDS
- AM ester MSDS

AM ester loading in rat cardiac myocytes[1] and rat vagal sensory neurons[2]

- Stock solution in DMSO
- Incubate cells in 3 to 10 µM Asante Calcium Red (AM) and 0.02% Pluronic F-127 for at least 45 minutes at room temperature
- Wash cells and maintain in medium for at least another 45 minutes to allow complete hydrolysis of AM esters by esterases.

Confocal images of emission ratiometric sensing of Ca^{2+} transients by Asante Calcium Red

- Rat cardiac myocytes (video)[1]
- Rat vagal sensory neurons[2]

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Product Specifications

Product Name	Asante Calcium Red (AM)	
Catalog Numbers	3000 and 3010	
MW	1200 g/mol	
K_d	~300-400 nM	
Description	red-orange solid	
TLC	60Å silica, 200 µm thickness	
Solvent	3:1 ethyl acetate/hexanes	
R_f	0.6	
Purity	> 95%	
HPLC	Waters™ XTerra® MS-C18, 4.6 x 100 mm	
Flow rate	1.4 mL / min	
Solvents	A = 0.01 M triethylammonium phosphate, pH 2.8 B = acetonitrile	
Solvent Gradient	65% B for 7 minutes (isocratic)	
Retention Time	4.71 minutes	
Detector Settings	254 nm	480 nm
Purity	> 95%	> 95%
Absorption Spectrum		
Solvent	Ethyl acetate	
λ_{max}	447 nm and 472 nm	
ε	23000 $M^{-1}cm^{-1}$	

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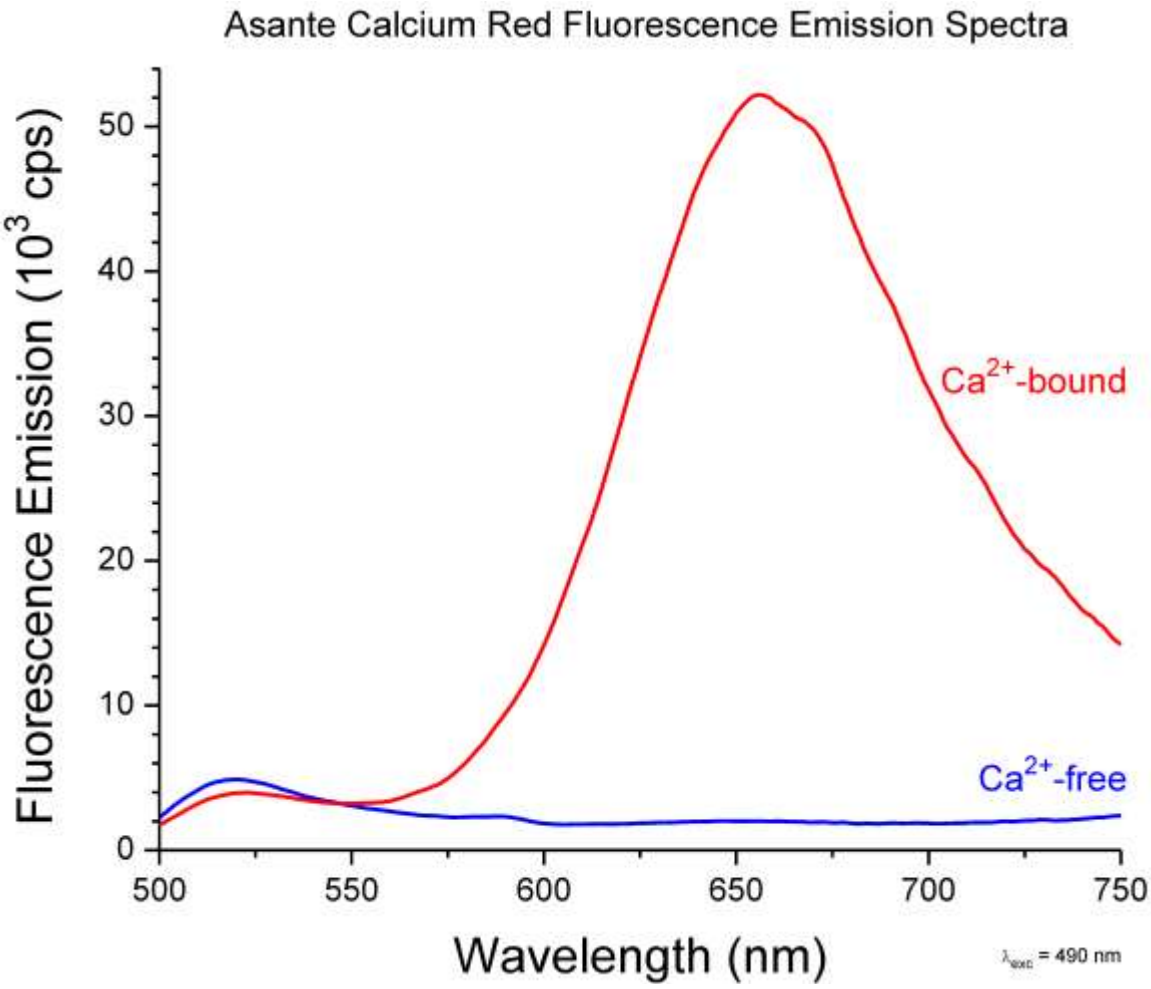
Product Specifications

Product Name	Asante Calcium Red (K^+ Salt)
Catalog Number	3020
MW	1000 g/mol
K_d	~300-400 nM
Description	Dark red solid
TLC	C_{18} on 60Å silica, 200 µm thickness
Solvent	7:3 methanol/brine
R_f	0.6
Purity	> 90%
HPLC	Waters™ XTerra® MS-C18, 4.6 x 100 mm
Flow rate	2 mL / min
Solvents	A = 0.01 M triethylammonium phosphate, pH 2.8 B = acetonitrile
Solvent Gradient	35% B for 10 min (isocratic)
Retention Time	6.76 minutes
Detector Settings	254 nm 460 nm
Purity	> 90% > 90%
Absorption Spectrum	
Solvent	Methanol
λ_{max}	555 nm
ε	45000 $M^{-1}cm^{-1}$
Ratiometric Emission Spectra	***(Figures 1)***
Solvent	10mM EGTA, 100mM KCl, 10mM MOPS, pH 7.2
λ_{ex}	488 nm
λ_{max}	517 nm (emission decreases slightly with increasing calcium) 650 nm (emission increases strongly with increasing calcium)
Non-Ratiometric Emission Spectra	***(Figure 2)***
Solvent	10mM EGTA, 100mM KCl, 10mM MOPS, pH 7.2
λ_{ex}	540 nm
λ_{max}	650 nm (emission increases strongly with increasing calcium)
Excitation Spectra	***(Figure 3)***
Solvent	10mM EGTA, 100mM KCl, 10mM MOPS, pH 7.2
λ_{em}	650 nm
λ_{max}	540 nm

***Figure 1*: Ratiometric emission traces of calcium-free and calcium-saturated Asante Calcium Red (K^+ Salt), lot 910a**



Note: Exciting at 488 nm (*Figure 1*) produces only 40% of the emission at 650 nm obtained from 540 nm excitation (*Figure 2*), so that adjustments for the reduced emission (i.e., higher dye concentration or increasing power to the excitation source) may be necessary.

***Figure 2*: Non-ratiometric emission traces of a calcium titration of Asante Calcium Red**

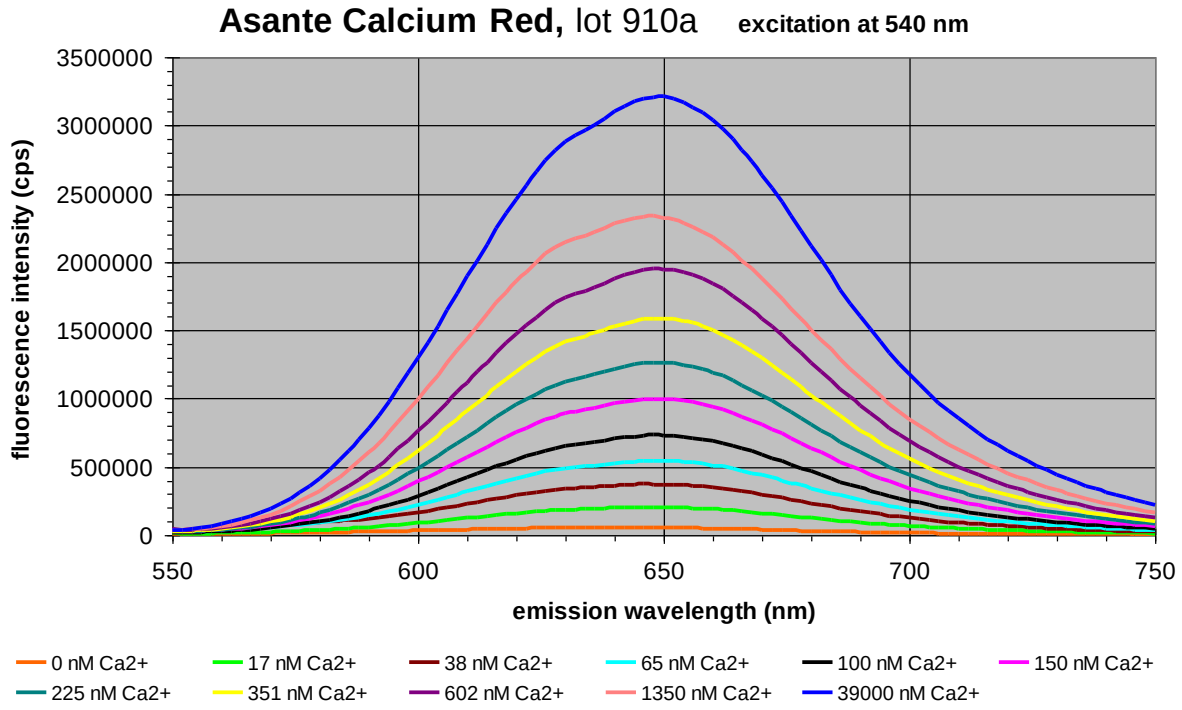
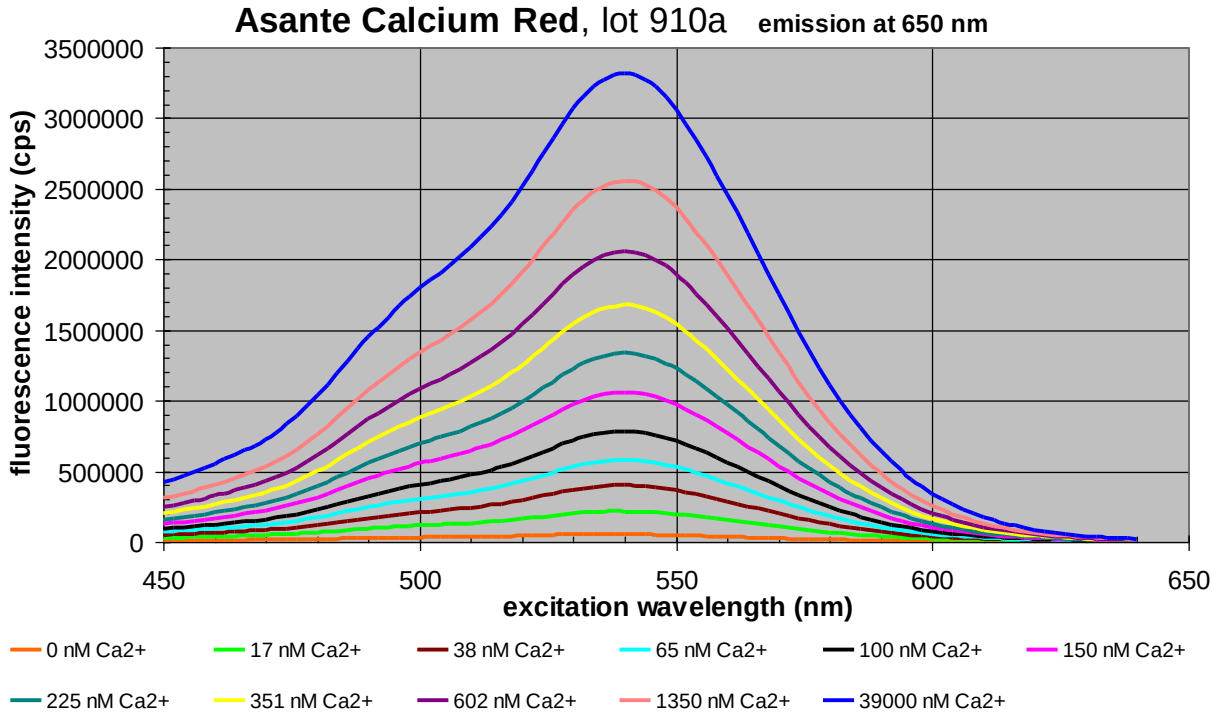


***Figure 3*: Excitation traces of a calcium titration of Asante Calcium Red (K+ Salt)**



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Material Safety Data Sheet

Catalog Number: 3000 and 3010

Product Name: Asante Calcium Red (AM)

Physical Characteristics: Red solid gum

Quantity: 500 µg / unit (3000) or 10 x 50 µg / unit (3010)

Odor: None

Fire and Explosion Hazards: None

Toxicology Dada: Not known

Emergency and First Aid: In case of contact with skin, eyes, or mouth, wash profusely with water. If swallowed, rinse mouth with water and seek medical advice.

For Further Information or in Emergency Contact the Manufacturer:

TEF Labs, Inc.
9415 Capitol View Dr
Austin, TX 78747
(512) 280-5223
support@teflabs.com

Safety Precautions and Control Measures:

Potentially harmful if inhaled or ingested. Do not get in eyes, on skin, or on clothing. Potential skin and eye irritant. Wash thoroughly after handling. Gloves should be worn when working with this material. Clean up procedure: wash with soap and water.

This material is for research and experimental applications only. It is not intended for food, drug, household, agricultural, or cosmetic use. All materials should be handled only by technically qualified individuals experienced in handling potentially hazardous chemicals. The above is correct to the best of our knowledge. The user should make independent decisions regarding completeness of the information based on all sources available. TEF Labs, Inc. shall not be held liable for any damage resulting from handling or from contact with the above product.

Date of writing or last revision: October 2009

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Material Safety Data Sheet

Catalog Number: 3020

Product Name: Asante Calcium Red (K^+ Salt)

Physical Characteristics: Red solid

Quantity: 250 µg / unit

Odor: None

Fire and Explosion Hazards: None

Toxicology Dada: Not known

Emergency and First Aid: In case of contact with skin, eyes, or mouth, wash profusely with water.
If swallowed, rinse mouth with water and seek medical advice.

For Further Information or in Emergency Contact the Manufacturer:

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Asante Calcium Red (AM) Loading

In rat cardiac myocytes[1]:

Acutely dissociated rat cardiac myocytes were incubated for 45 min at room temperature with Tyrode's solution containing 10 μM Asante Calcium Red (AM) and 0.02% (wt/vol) Pluronic F-127. The cells were then transferred to fresh Tyrode's solution and allowed to stand for an additional 45 min to ensure complete hydrolysis of the AM ester.

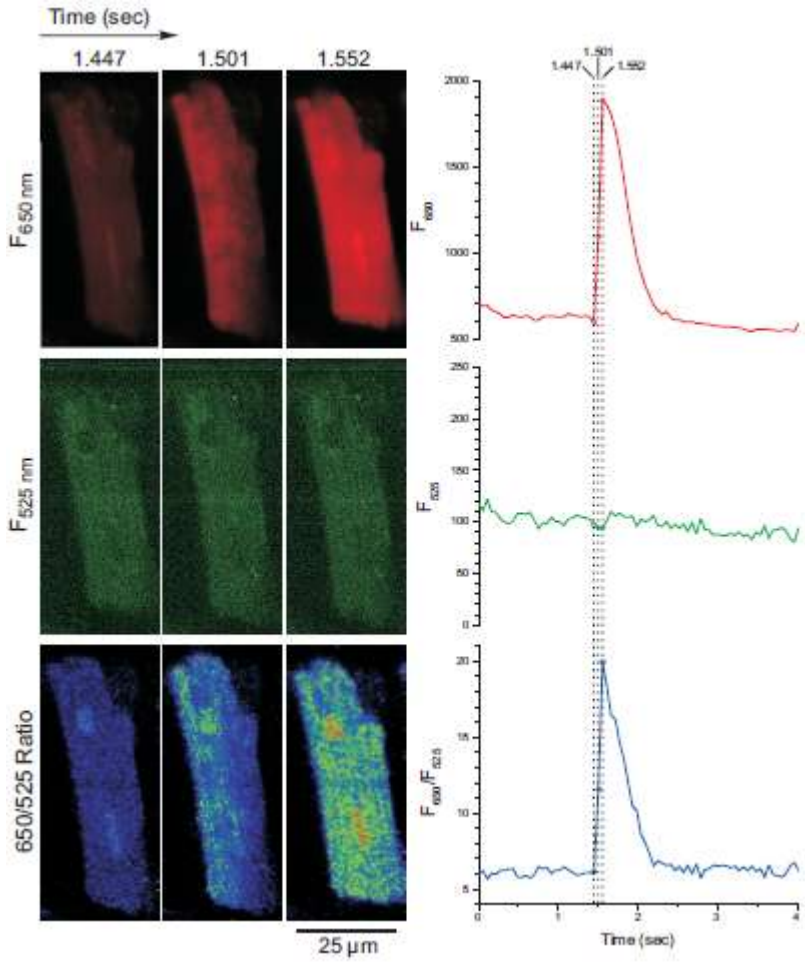
In rat vagal sensory neurons[2]:

The inferior vagal (nodose) ganglia from a Sprague-Dawley rat were dissociated enzymatically. The yield of nodose neurons was suspended in Leibovitz L-15 medium supplemented with 10% (v/v) fetal bovine serum and penicillin-streptomycin and plated onto No. 1 glass coverslips.

Neurons were incubated for 50 min at room temperature with 3 μM Asante Calcium Red (AM) ester. Thereafter, the cells were washed and maintained in fresh L-15 medium for 40 min to permit intracellular enzymatic hydrolysis of the AM ester to proceed to completion.

Confocal image of emission ratiometric sensing of Ca^{2+} transients by Asante Calcium Red

In rat cardiac myocytes[1]:

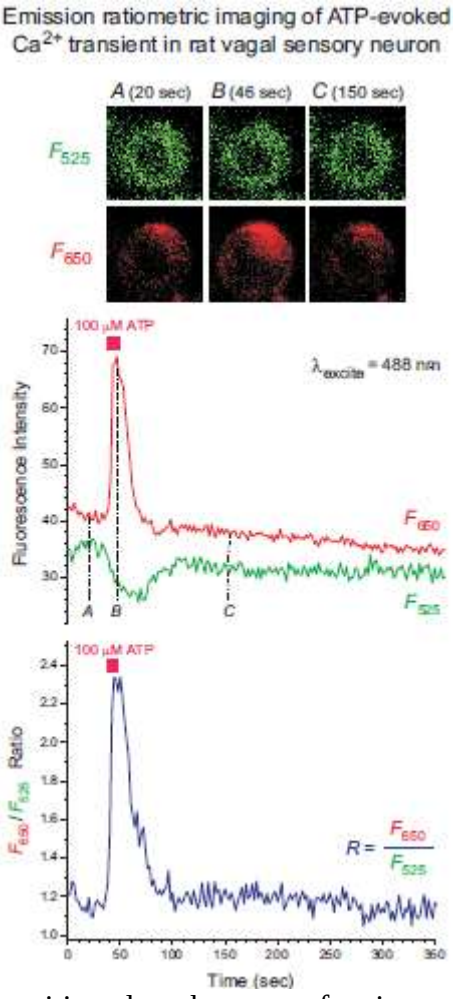


Myocytes loaded with Asante Calcium Red were superfused with Tyrode' solution and imaged on a Zeiss 5 Live laser scanning confocal microscope. With 488-nm excitation, fluorescence images were acquired at 525 nm and 650 nm at 19 frames per second. The background fluorescence for each image was defined as the mean intensity value in a region-of-interest located near the cell but outside the cell contour. Fluorescence images were acquired at 650 and 525 nm (F650 nm and F525 nm) and ratioed. Fluorescence intensities and the 650/525 fluorescence ratio as functions of time are shown in the line plots at right. Electric field stimulation was used to evoke a Ca2+ transient and contraction. At left, fluorescence and ratio images are shown for three time points: a) 1.447 sec, immediately before field stimulation; b) 1.501 sec, immediately following stimulation, during the rising phase of the Ca2+ transient; and c) 1.662 sec, at the peak of the Ca2+ transient.

Rat cardiac myocyte loading of the AM ester

Confocal images of emission ratiometric sensing of Ca^{2+} transients by Asante Calcium Red

In rat vagal sensory neurons[2]:



Indicator-loaded neurons were positioned on the stage of an inverted microscope (Axiovert 100 M, Zeiss) and superfused with Locke solution (equilibrated with a mixture of 5% CO_2 and 95% O_2). Confocal imaging microscopy was performed with the LSM 510 system (Zeiss) through a ×40 objective (N.A. 1.2; oil-immersion) at a frame rate of 0.5 Hz. Excitation was at 488-nm; a 545-nm dichroic mirror separated the fluorescence emission into two components: a short-wavelength component (designated F_{525}) that passed through a 500-550-nm band-pass filter, and a long-wavelength component (F_{650}) that passed through a 560-nm long-pass filter. Neurons were stimulated with a 10-sec pulse of 100 µM ATP delivered through the superfusate.

Rat vagal sensory neuron loading of AM ester

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ACCREDITATION

AM ester loading conditions and confocal imaging of sensing of calcium transients by Asante Calcium Red were contributed by:

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