

Classic Calcium Detection Reagents

Calcium acts as a universal second messenger in a variety of cells. Numerous functions of all types of cells are regulated by Ca^{2+} to a greater or lesser degree, thus calcium measurement is critical for numerous biological investigations. Since the 1920s, scientists have attempted to measure Ca^{2+} , but few were successful due to limited availability of Ca^{2+} probes. The first reliable measurement of Ca^{2+} was performed by Ridgway and Ashley by injecting the photoprotein aequorin into the giant muscle fiber of the barnacle. Subsequently, in the 1980s, Tsien and colleagues produced a variety of fluorescent indicators. Among them Indo-1, Fura-2, Fluo-3 and Rhod-2 have been the most valuable dyes for measuring Ca^{2+} with a fluorescence instrument.

Fluorescent probes that show spectral responses upon binding Ca^{2+} have enabled researchers to investigate changes in intracellular free Ca^{2+} concentrations by using fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers. Most of these fluorescent indicators are derivatives of BAPTA chelators that incorporate a PET system responsive to calcium. FLIPR® and FlexStation™ instruments of Molecular Devices Corp., FDSS of Hamamatsu Corp. and NOVOstar of BMG Technologies have enabled high throughput measurement of calcium for GPCR and ion channel research. There are quite a few factors that need be considered when selecting a fluorescent Ca^{2+} indicator. These include:

- *Spectral Properties:* For UV excitation, Indo-1 and Fura-2 are widely used. Fluo-3 is preferred for 488 nm excitation while Rhod-2 and X-rhod are used for red emissions.
- *Measurement Mode:* Ion indicators that exhibit spectral shifts upon ion binding can be used for ratiometric measurements of Ca^{2+} concentration, which are essentially independent of uneven dye loading, cell thickness, photobleaching effects and dye leakage. Excitation and emission wavelength preferences depend on the type of instrumentation being used, as well as on sample autofluorescence and on the presence of other fluorescent or photoactivatable probes in the experiment. Indo-1 and Fura-2 are primary choice for ratiometric measurements while Fluo-3 and Rhod-2 are predominantly used for single wavelength measurements.
- *Permeability of Ca^{2+} Indicators (salt or AM ester):* The salt forms are typically loaded into cells by microinjection, microprojectile bombardment or electroporation, or used for extracellular assays. In contrast, the cell-permeant acetoxymethyl (AM) esters can be passively loaded into cells, where they are cleaved to cell-impermeant products by intracellular esterases.
- *Dissociation Constant (K_d):* The desired indicators must have a proper K_d compatible with the Ca^{2+} concentration range of interest. Indicators have a detectable response in the concentration range from approximately $0.1\mu\text{M}$ K_d to $10\mu\text{M}$ K_d . The K_d values of Ca^{2+} indicators are dependent on many factors, including pH, temperature, ionic strength, viscosity, protein binding and the presence of Mg^{2+} and other ions. Consequently, K_d values for intracellular indicators are usually significantly higher than corresponding values measured in cell-free solutions.

UV-Excitable Calcium Indicators

Among the UV-excitable calcium indicators, Fura-2 and Indo-1 are most commonly used. Fura-2 is excitation-ratioable while Indo-1 is emission-ratioable. Fura-2 is preferred for ratio-imaging microscopy, in which it is more practical to change excitation wavelengths than emission wavelengths. Upon binding Ca^{2+} , Fura-2 exhibits an absorption shift that can be observed by scanning the excitation spectrum between 300 and 400 nm, while monitoring the emission at ~510 nm. In contrast, Indo-1 is the preferred dye for flow cytometry, where it is more practical to use a single laser for excitation (usually the 351–364 nm spectral lines of the argon-ion laser).

Table 1. UV-Excitable Fluorescent Calcium Indicators

#CAT	Product Name	Unit	MW	Ex (nm)	Em (nm)
22006	Calcein blue	250 mg	321.28	360	445
22017	Calcein blue, AM	1 mg	465.41	360	445
22012	CytoCalcein Violet 450, AM *Excited at 405 nm*	1 mg	~400	408	450
21020	Fura-2, AM	1 mg	1001.86	370	476
21024	Fura-2, AM *1mM solution in anhydrous DMSO*	1 mL	1001.86	370	476

21022	Fura-2, AM *Bulk packaging*	50 mg	1001.86	370	476
21023	Fura-2, AM *Custom packaging*	1 mg	1001.86	370	476
21021	Fura-2, AM *UltraPure Grade*	1 mg	1001.86	370	476
21025	Fura-2, pentapotassium salt	1 mg	832.00	363	512
21026	Fura-2, pentasodium salt	1 mg	751.45	363	512
21030	Indo-1, AM	1 mg	1009.91	346	475
21038	Indo-1, AM *1 mM solution in anhydrous DMSO*	1 mL	1009.91	346	475
21033	Indo-1, AM *Bulk packaging*	50 mg	1009.91	346	475
21036	Indo-1, AM *Custom packaging*	20x50 µg	1009.91	346	475
21032	Indo-1, AM *UltraPure Grade*	1 mg	1009.91	346	475
21040	Indo-1, pentapotassium salt	1 mg	840.05	346	475
21044	Indo-1, pentasodium salt	1 mg	759.52	346	475
21050	Quin-2, AM	1 mg	829.76	346	475
21052	Quin-2, tetrapotassium salt	5 mg	693.87	353	495

*Note: Spectral data of esterase-hydrolyzed product.

Visible Light-Excitable Calcium Indicators

Among the visible light-excitable calcium indicators, Fluo-3 and Rhod-2 are most commonly used. Fluo-3 indicators are widely used in flow cytometry and confocal laser-scanning microscopy. More recently, Fluo-3, AM has been extensively used in cell-based high-throughput screening assays for functional GPCR assays. Fluo-3 is essentially nonfluorescent unless bound to Ca^{2+} and exhibits a quantum yield at saturating Ca^{2+} of ~0.14 and a K_d for Ca^{2+} of 390 nM.

The long-wavelength Rhod-2 is valuable alternative Ca^{2+} indicators to Fluo-3 for experiments in cells and tissues that have high levels of autofluorescence. Rhod-5N has a lower binding affinity for Ca^{2+} than any other BAPTA-based indicator ($K_d = \sim 320 \mu\text{M}$) and is suitable for Ca^{2+} measurements from 10 μM to 1 mM. Like the parent Rhod-2 indicator, Rhod-5N is essentially nonfluorescent in the absence of divalent cations and exhibits strong fluorescence enhancement with no spectral shift upon binding Ca^{2+} . Both the Fluo and Rhod indicators are available as cell-impermeant potassium salts or as cell-permeant AM esters.

Table 2. Visible Light-Excitable Fluorescent Calcium Indicators

#CAT	Product Name	Unit	MW	Ex (nm)	Em (nm)
22002	Calcein, AM	1 mg	994.86	495	515
22003	Calcein, AM *UltraPure grade*	1 mg	994.86	495	515
22004	Calcein, AM *UltraPure grade*	20x50 µg	994.86	495	515
21010	Fluo-3, AM	1 mg	1129.85	506	526
21014	Fluo-3, AM *1 mM solution in anhydrous DMSO*	1 mL	1129.85	506	526
21012	Fluo-3, AM *Bulk package*	50 mg	1129.85	506	526
21013	Fluo-3, AM *Custom packaging*	20x50 µg	1129.85	506	526
21011	Fluo-3, AM *UltraPure grade*	1 mg	1129.85	506	526
21018	Fluo-3, pentaammonium salt	1 mg	854.69	506	526
21017	Fluo-3, pentapotassium salt	1 mg	959.98	506	526
21016	Fluo-3, pentasodium salt	1 mg	879.44	506	526
21090	Quest Fluo-8H™, AM	1 mg	~1100	490	514
21091	Quest Fluo-8H™, AM	10x50 µg	~1100	490	514

21095	Quest Fluo-8H™, sodium salt	10x50 µg	~800	490	514
21096	Quest Fluo-8L™, AM	1 mg	~1100	490	514
21097	Quest Fluo-8L™, AM	10x50 µg	~1100	490	514
21098	Quest Fluo-8L™, sodium salt	10x50 µg	~800	490	514
21080	Quest Fluo-8™, AM	1 mg	~1000	490	514
21081	Quest Fluo-8™, AM	5x50 µg	~1000	490	514
21082	Quest Fluo-8™, AM	10x50 µg	~1000	490	514
21083	Quest Fluo-8™, AM	20x50 µg	~1000	490	514
21088	Quest Fluo-8™, sodium salt	10x50 µg	~800	490	514
21120	Quest Rhod-4™, AM	1 mg	~1000	530	555
21121	Quest Rhod-4™, AM	5x50 µg	~1000	530	555
21122	Quest Rhod-4™, AM	10x50 µg	~1000	530	555
21123	Quest Rhod-4™, AM	20x50 µg	~1000	530	555
21128	Quest Rhod-4™, sodium salt	5x50 µg	~800	530	555
21060	Rhod-2, AM	1 mg	1123.96	549	578
21062	Rhod-2, AM *UltraPure Grade*	1 mg	1123.96	549	578
21063	Rhod-2, AM *UltraPure Grade* *Bulk packaging*	50 mg	1123.96	549	578
21064	Rhod-2, AM *UltraPure Grade*	20x50 µg	1123.96	549	578
21067	Rhod-2, tripotassium salt	1 mg	869.05	549	578
21068	Rhod-2, trisodium salt	1 mg	820.73	549	578
21070	Rhod-5N, AM	1 mg	1154.92	551	577
21072	Rhod-5N, tripotassium salt	1 mg	900.02	551	577
36314	Screen Quest™ Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium* *1 Plate*	1 kit	N/A	490	514
36315	Screen Quest™ Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium* *10 Plates*	1 kit	N/A	490	514
36316	Screen Quest™ Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium* *10x10 Plates*	1 kit	N/A	490	514
36307	Screen Quest™ Fluo-8 NW Calcium Assay Kit *Medium Removal* *1 Plate*	1 kit	N/A	490	514
36308	Screen Quest™ Fluo-8 NW Calcium Assay Kit *Medium Removal* *10 Plates*	1 kit	N/A	490	514
36309	Screen Quest™ Fluo-8 NW Calcium Assay Kit *Medium Removal* *10x10 Plates*	1 kit	N/A	490	514
36334	Screen Quest™ Rhod-4 NW Calcium Assay Kit *1% FBS Growth Medium* *10 plates*	1 kit	N/A	530	555
36333	Screen Quest™ Rhod-4 NW Calcium Assay Kit *1% FBS Growth Medium* *1 plate*	1 kit	N/A	530	555
36335	Screen Quest™ Rhod-4 NW Calcium Assay Kit *1% FBS Growth Medium* *10x10 plates*	1 kit	N/A	530	555
36330	Screen Quest™ Rhod-4 NW Calcium Assay Kit *Medium Removal* *1 plate*	1 kit	N/A	530	555
36331	Screen Quest™ Rhod-4 NW Calcium Assay Kit *Medium Removal* *10 plate*	1 kit	N/A	530	555
36332	Screen Quest™ Rhod-4 NW Calcium Assay Kit *Medium Removal* *10x10 plate*	1 kit	N/A	530	555

*Note: Spectral data of esterase-hydrolyzed product.

Coelenterazine and Its Synthetic Analogs for Luminescent Calcium Detection

The aequorin complex comprises a 22,000-dalton apoaequorin protein, molecular oxygen and the luminophore coelenterazine. When three Ca^{2+} ions bind to this complex, coelenterazine is oxidized to coelenteramide, with a concomitant release of carbon dioxide and blue light. The approximately third-power dependence of aequorin's bioluminescence on Ca^{2+} concentration allows the measurement of Ca^{2+} concentrations with a broad detection range from $\sim 0.1 \mu\text{M}$ to $>100 \mu\text{M}$. Unlike fluorescent Ca^{2+} indicators, Ca^{2+} -bound aequorin can be detected without illuminating the sample, thereby eliminating interference from autofluorescence.

ABD Bioquest offers coelenterazine and several synthetic coelenterazine analogs for reconstituting aequorin in cells that have been transfected with apoaequorin cDNA. In addition to native coelenterazine, we also offer a few derivatives of coelenterazine that confer different Ca^{2+} affinities and spectral properties on the aequorin complex. Recombinant apoaequorin reconstituted with coelenterazine *hcp* is reported to have the best luminescence overall, with both a high quantum yield and a fast response time. However, intracellular reconstitution of aequorin from coelenterazine analogs can be relatively slow. Aequorins containing the *cp*, *f* or *h* form of coelenterazine exhibit 10–20 times stronger luminescence than that of apoaequorin reconstituted with native coelenterazine. Coelenterazine *cp* and *h* has been used in HTS screening assay for GPCRs.

ABD Bioquest also offers two luminescent calcium assay kits. These two kits use a highly calcium-sensitive and membrane-permeable coelenterazine analog as a calcium indicator for the cells that are transfected with apoaequorin gene. Our coelenterazine-based kit is much more sensitive than the fluorescence-based calcium assay kits (such as Fluo-4, Fluo-3, Calcium-3 and Calcium-4). This kit provides an optimized assay method for monitoring G-protein-coupled receptors (GPCRs) and calcium channels. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation.

Table 3. Luminescent Calcium Indicators

#CAT	Product Name	Unit	MW	Ex (nm)	Em (nm)	RL*	HRT* (ms)
21150	Coelenterazine *UltraPure grade*	250 μg	423.46	429	466	1	6-30
21151	Coelenterazine cp *UltraPure grade*	250 μg	415.48	430	442	28	2-5
21152	Coelenterazine f *UltraPure grade*	250 μg	425.45	437	472	20	6-30
21153	Coelenterazine h *UltraPure grade*	250 μg	407.46	437	466	16	6-30
21154	Coelenterazine hcp *UltraPure grade*	250 μg	399.48	433	445	500	2-5
21155	Coelenterazine n *UltraPure grade*	250 μg	457.52	431	468	0.15	6-30
36305	Screen Quest™ Luminometric Calcium Assay Kit *10 Plates	1 kit	N/A	N/A	N/A	N/A	N/A

*Notes: a). RL = Relative luminescence; HRT = half rise time in milli seconds;

b). Data from O. Shimomura, *et al.* (1993). The relative rate of aequorin regeneration from apoaequorin and coelenterazine analogues. *Biochem J* **296** (Pt 3), 549-51.

Non-Fluorescent Reagents for Calcium Detection

Intracellular calibration of Ca^{2+} indicators may be achieved either by manipulating Ca^{2+} levels inside cells using an ionophore or by releasing the indicator into the surrounding medium of known Ca^{2+} concentration via detergent lysis of the cells. Besides the fluorescent and luminescent calcium detection reagents, we also offer several non-luminescent compounds for measuring and manipulating intracellular and extracellular Ca^{2+} .

Table 4. Non-Fluorescent Calcium Detection Reagents

#CAT	Product Name	Unit	MW
21001	BAPTA, AM	25 mg	764.68
21002	BAPTA, AM *UltraPure Grade*	25 mg	764.68
21003	BAPTA, tetrapotassium salt	100 mg	628.79
21004	BAPTA, tetrasodium salt	100 mg	564.36

21005	EGTA AM	10 mg	668.6
21006	EGTA AM *10 mM DMSO solution*	1 mL	668.6
21008	EGTA tetrasodium salt *10 mM aqueous solution*	10 mL	468.28
21007	EGTA tetrasodium salt *UltraPure Grade*	1 g	468.28
20053	Pluronic® F-127 *10% solution in water*	10 mL	N/A
20052	Pluronic® F-127 *20% solution in DMSO*	10 mL	N/A
20050	Pluronic® F-127 *Cell culture tested *	10 g	N/A
20060	Probenecid *Cell culture tested*	10x150 mg	285.36
20061	Probenecid *Water-soluble*	10x150 mg	307.34

References

1. Mattson MP. (2007) Calcium and neurodegeneration. *Aging Cell*, 6, 337.
2. Gaspers LD, Thomas AP. (2005) Calcium signaling in liver. *Cell Calcium*, 38, 329.
3. Martin VV, Beierlein M, Morgan JL, Rothe A, Gee KR. (2004) Novel fluo-4 analogs for fluorescent calcium measurements. *Cell Calcium*, 36, 509.
4. Rudolf R, Mongillo M, Rizzuto R, Pozzan T. (2003) Looking forward to seeing calcium. *Nat Rev Mol Cell Biol*, 4, 579.
5. Yip KP, Kurtz I. (2002) Confocal fluorescence microscopy measurements of pH and calcium in living cells. *Methods Cell Biol*, 70, 417.
6. do Ceu Monteiro M, Sansonetty F, Goncalves MJ, O'Connor JE. (1999) Flow cytometric kinetic assay of calcium mobilization in whole blood platelets using Fluo-3 and CD41. *Cytometry*, 35, 302.
7. Takahashi A, Camacho P, Lechleiter JD, Herman B. (1999) Measurement of intracellular calcium. *Physiol Rev*, 79, 1089.
8. DeBernardi MA, Brooker G. (1998) Simultaneous fluorescence ratio imaging of cyclic AMP and calcium kinetics in single living cells. *Adv Second Messenger Phosphoprotein Res*, 32, 195.
9. Silver RB. (1998) Ratio imaging: practical considerations for measuring intracellular calcium and pH in living tissue. *Methods Cell Biol*, 56, 237.
10. Su ZL, Li N, Sun YR, Yang J, Wang IM, Jiang SC. (1998) [Monitoring calcium in outer hair cells with confocal microscopy and fluorescence ratios of fluo-3 and fura-red]. *Shi Yan Sheng Wu Xue Bao*, 31, 323.
11. Tretyn A, Kado RT, Kendrick RE. (1997) Loading and localization of Fluo-3 and Fluo-3/AM calcium indicators in sinapis alba root tissue. *Folia Histochem Cytobiol*, 35, 41.
12. Perez-Terzic C, Stehno-Bittel L, Clapham DE. (1997) Nucleoplasmic and cytoplasmic differences in the fluorescence properties of the calcium indicator Fluo-3. *Cell Calcium*, 21, 275.
13. Greimers R, Trebak M, Moutschen M, Jacobs N, Boniver J. (1996) Improved four-color flow cytometry method using fluo-3 and triple immunofluorescence for analysis of intracellular calcium ion ([Ca²⁺]_i) fluxes among mouse lymph node B- and T-lymphocyte subsets. *Cytometry*, 23, 205.
14. Bolsover S. (1995) Using fluorescence to probe calcium signalling mechanisms. *Biochem Soc Trans*, 23, 627.
15. Thomas AP, Renard-Rooney DC, Hajnoczky G, Robb-Gaspers LD, Lin C, Rooney TA. (1995) Subcellular organization of calcium signalling in hepatocytes and the intact liver. *Ciba Found Symp*, 188, 18.
16. Baus E, Urbain J, Leo O, Andris F. (1994) Flow cytometric measurement of calcium influx in murine T cell hybrids using Fluo-3 and an organic-anion transport inhibitor. *J Immunol Methods*, 173, 41.
17. Felder CC, Singer-Lahat D, Mathes C. (1994) Voltage-independent calcium channels. Regulation by receptors and intracellular calcium stores. *Biochem Pharmacol*, 48, 1997.
18. Trautmann A, Tan YP. (1993) Calcium imaging. *Blood Cells*, 19, 133.
19. Fumagalli G, Zacchetti D, Lorenzon P, Grohovaz F. (1991) Fluorimetric approaches to the study of calcium transients in living cells. *Cytotechnology*, 5 Suppl 1, 99.
20. Michelangeli F. (1991) Fluo-3 an ideal calcium indicator for measuring calcium fluxes in SR and ER. *Biochem Soc Trans*, 19, 183S.
21. Roe MW, Lemasters JJ, Herman B. (1990) Assessment of Fura-2 for measurements of cytosolic free calcium. *Cell Calcium*, 11, 63.
22. Saavedra-Molina A, Uribe S, Devlin TM. (1990) Control of mitochondrial matrix calcium: studies using fluo-3 as a fluorescent calcium indicator. *Biochem Biophys Res Commun*, 167, 148.