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 to 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 to be considered when selecting a fluorescent Ca^{2+} indicator.

- *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 the sample autofluorescence and on the presence of other fluorescent or photoactivatable probes in the experiment. Indo-1 and Fura-2 are primary choices 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 μM K_d to 10 μM 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 to 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)[1]	Em (nm)[1]
21908	Calcein UltraBlue™, AM	10 X 50 µg	788.65	360	445
21909	Calcein UltraBlue™ Sodium Salt	1 mg	566.35	360	445
22006	Calcein blue	25 mg	321.28	360	445
22007	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	340/380	510
21022	Fura-2, AM *Bulk packaging*	50 mg	1001.86	340/380	510

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21023	Fura-2, AM *Custom packaging*	1 mg	1001.86	340/380	510
21021	Fura-2, AM *UltraPure Grade*	1 mg	1001.86	340/380	510
21025	Fura-2, pentapotassium salt	1 mg	832.00	340/380	510
21026	Fura-2, pentasodium salt	1 mg	751.45	340/380	510
21055	Fura-8™, AM	1 mg	1102.95	354/415	524
21056	Fura-8™, AM	10 X 50 µg	1102.95	354/415	524
21057	Fura-8™, pentapotassium salt	1 mg	816.01	354/415	524
21058	Fura-8™, pentasodium salt	1 mg	751.58	354/415	524
20602	Fura- 8FF™, AM	10 X 50 µg	973.86	354/415	524
20621	Fura-8FF™, pentapotassium salt	10 X 50 µg	837.97	354/415	524
21027	Fura-FF, AM [Fura-2FF, AM]	10 X 50 µg	1023.80	363	512
21028	Fura-FF, pentapotassium salt [Fura-2FF, pentapotassium salt]	10 X 50 µg	853.94	363	512
21030	Indo-1, AM	1 mg	1009.91	340	400/500
21033	Indo-1, AM *Bulk packaging*	50 mg	1009.91	340	400/500
21036	Indo-1, AM *Custom packaging*	20×50 µg	1009.91	340	400/500
21032	Indo-1, AM *UltraPure Grade*	1 mg	1009.91	340	400/500
21040	Indo-1, pentapotassium salt	1 mg	840.05	340	400/500
21044	Indo-1, pentasodium salt	1 mg	759.52	340	400/500
21050	Quin-2, AM	1 mg	829.76	350/330	495
21052	Quin-2, tetrapotassium salt	5 mg	693.87	350/330	495

[1]*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 screenings 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 a valuable Ca^{2+} indicators alternative 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 = ~320 µM) 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 to Ca^{2+}. Both the Fluo and Rhod indicators are available as cell-impermeant potassium or sodium salts or as cell-permeant AM esters.

Table 2. Visible Light-Excitable Fluorescent Calcium Indicators

Cat. #	Product Name	Unit	MW	Ex (nm)[2]	Em (nm)[2]
21130	Cal-520®, AM	10 x 50 µg	1102.95	492	514
21131	Cal-520®, AM	1 mg	1102.95	492	514
21135	Cal-520™, Sodium salt	10 x 50 µg	840.54	492	514
21136	Cal-520™, Sodium salt	1 mg	840.54	492	514
21140	Cal-520™, Potassium salt	10 x 50 µg	921.08	492	514
21141	Cal-520™, Potassium salt	1 mg	921.08	492	514
21142	Cal-520FF™, AM	1 mg	1138.92	492	514
21143	Cal-520FF™, AM	10 x 50 µg	1138.92	492	514
21144	Cal-520FF™, potassium salt	10 x 50 µg	957.06	492	514
20510	Cal-590™ AM	5 x 50 µg	1266.81	573	588

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20511	Cal-590™ AM	10 x 50 µg	1266.81	573	588
20512	Cal-590™ AM	1 mg	1266.81	573	588
20515	Cal-590™, sodium salt	5 x 50 µg	1026.38	573	588
20518	Cal-590™, potassium salt	5 x 50 µg	1123.03	573	588
20530	Cal-630™ AM	5 x 50 µg	1282.89	608	626
20531	Cal-630™ AM	10 x 50 µg	1282.89	608	626
20532	Cal-630™ AM	1 mg	1282.89	608	626
20535	Cal-630™, sodium salt	5 x 50 µg	1042.45	608	626
20538	Cal-630™, potassium salt	5 x 50 µg	1139.11	608	626
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
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*	20 x 50 µg	994.86	495	515
20500	Cal Green™ 1, hexapotassium salt	10 x 50 µg	1147.18	506	531
20501	Cal Green™ 1, AM [Equivalent to Calcium Green-1, AM]	10 x 50 µg	1290.96	506	531
20502	Cal Green™ 1, AM [Equivalent to Calcium Green-1, AM]	1 mg	1290.96	506	531
20588	Cal Red™ R525/650 potassium salt	5 x 50 µg	~1000	492	525/650
20590	Cal Red™ R525/650 AM	1 mg	~1100	492	525/650
20591	Cal Red™ R525/650 AM	10 x 50 µg	~1100	492	525/650
21010	Fluo-3, AM	1 mg	1129.85	506	526
21012	Fluo-3, AM *Bulk package*	50 mg	1129.85	506	526
21013	Fluo-3, AM *Custom packaging*	20 x 50 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
21014	Fluo-3FF, AM *UltraPure grade* *Cell permeant*	10 x 50 µg	1151.80	506	526
21019	Fluo-3FF, pentapotassium salt	1 mg	981.94	506	526
20550	Fluo-4 AM *Ultrapure Grade*	1 mg	1096.95	494	516
20551	Fluo-4 AM *Ultrapure Grade*	10 x 50 µg	1096.95	494	516
20552	Fluo-4 AM *Ultrapure Grade*	5 x 50 µg	1096.95	494	516
20555	Fluo-4, Pentapotassium Salt	1 mg	927.08	494	516
20556	Fluo-4, Pentapotassium Salt	10 x 50 µg	927.08	494	516
21054	BTC, AM	1 mg	979.92	401/464	529
21053	BTC, tetrapotassium salt	1 mg	844.02	401/464	529
21080	Fluo-8®, AM	1 mg	1046.93	494	517
21081	Fluo-8®, AM	5 x 50 µg	~1000	494	517
21082	Fluo-8®, AM	10 x 50 µg	~1000	494	517
21083	Fluo-8®, AM	20 x 50 µg	~1000	494	517

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21086	Fluo-8®, sodium salt	1 mg	796.53	494	517
21087	Fluo-8®, potassium salt	1 mg	877.07	494	517
21088	Fluo-8®, sodium salt	10 x 50 µg	796.53	494	517
21089	Fluo-8®, potassium salt	10 x 50 µg	877.07	494	517
21910	Fluo-8H™, AM	1 mg	1074.98	494	517
21091	Fluo-8H™, AM	10 x 50 µg	1074.98	494	517
21095	Fluo-8H™, sodium salt	10 x 50 µg	802.60	494	517
21096	Fluo-8L™, AM	1 mg	1078.95	494	517
21097	Fluo-8L™, AM	10 x 50 µg	1078.95	494	517
21098	Fluo-8L™, sodium salt	10 x 50 µg	828.54	494	517
21099	Fluo-8L™, sodium salt	1 mg	828.54	494	517
21100	Fluo-8L™, potassium salt	10x50 µg	909.09	494	517
21101	Fluo-8L™, potassium salt	1 mg	909.09	494	517
21102	Fluo-8FF™, potassium salt	10x50 µg	913.05	494	517
21103	Fluo-8FF™, potassium salt	1 mg	913.05	494	517
21104	Fluo-8FF™, AM	10x50 µg	1082.91	494	517
21105	Fluo-8FF™, AM	1 mg	1082.91	494	517
21118	Rhod-4™, sodium salt	1 mg	815.64	524	551
21119	Rhod-4™, potassium salt	1 mg	880.07	524	551
21120	Rhod-4™, AM	1 mg	1015.96	524	551
21121	Rhod-4™, AM	5 x 50 µg	1015.96	524	551
21122	Rhod-4™, AM	10 x 50 µg	1015.96	524	551
21123	Rhod-4™, AM	20 x 50 µg	1015.96	524	551
21128	Rhod-4™, sodium salt	5 x 50 µg	815.64	524	551
21129	Rhod-4™, potassium salt	5 x 50 µg	880.07	524	551
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*	20 x 50 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
21075	Rhod-FF, tripotassium salt	1 mg	891	549	578
21076	Rhod-FF, tripotassium salt	10 x 50 µg	891	549	578
21077	Rhod-FF, AM	1 mg	1145.9	549	578
21078	Rhod-FF, AM	10 x 50 µg	1145.9	549	578
36314	Screen Quest™ Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium* *1 Plate*	1 plate	N/A	490	525
36315	Screen Quest™ Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium* *10 Plates*	10 plates	N/A	490	525
36316	Screen Quest™ Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium* *10×10 Plates*	100 plates	N/A	490	525
36307	Screen Quest™ Fluo-8 NW Calcium Assay Kit *Medium Removal* *1 Plate*	1 plate	N/A	490	525
36308	Screen Quest™ Fluo-8 NW Calcium Assay Kit *Medium Removal* *10 Plates*	10 plates	N/A	490	525
36309	Screen Quest™ Fluo-8 NW Calcium Assay Kit *Medium Removal* *10×10 Plates*	100 plates	N/A	490	525

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36320	Screen Quest™ Fura-2 No Wash Calcium Assay Kit	10 plates	N/A	340/380	510
36321	Screen Quest™ Fura-2 No Wash Calcium Assay Kit	100 plates	N/A	340/380	510
36333	Screen Quest™ Rhod-4 NW Calcium Assay Kit *1% FBS Growth Medium * *1 plate*	1 plate	N/A	530	590
36334	Screen Quest™ Rhod-4 NW Calcium Assay Kit *1% FBS Growth Medium* *10 plates*	10 plates	N/A	530	590
36335	Screen Quest™ Rhod-4 NW Calcium Assay Kit *1% FBS Growth Medium* *10×10 plates*	100 plates	N/A	530	590
36330	Screen Quest™ Rhod-4 NW Calcium Assay Kit *Medium Removal* *1 plate*	1 plate	N/A	530	590
36331	Screen Quest™ Rhod-4 NW Calcium Assay Kit *Medium Removal* *10 plate*	10 plates	N/A	530	590
36332	Screen Quest™ Rhod-4 NW Calcium Assay Kit *Medium Removal* *10×10 plate*	100 plates	N/A	530	590
36300	Screen Quest™ 10X cell staining buffer with Phenol Red Plus™	10 plates	N/A	N/A	N/A
36301	Screen Quest™ 10X calcium assay buffer with Phenol Red Plus™	10 plates	N/A	N/A	N/A

[2]*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 ranging from ~0.1 µM to >100 µM. Unlike fluorescent Ca^{2+} indicators, Ca^{2+}-bound aequorin can be detected without illuminating the sample, thereby eliminating interference from autofluorescence.

AAT 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* have been used in HTS screening assay for GPCRs.

AAT 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[3]	HRT[3] (ms)
21150	Coelenterazine	250 µg	423.46	429	466	1	6-30
21151	Coelenterazine cp	250 µg	415.48	430	442	28	2-5
21152	Coelenterazine f	250 µg	425.45	437	472	20	6-30
21153	Coelenterazine h	250 µg	407.46	437	466	16	6-30
21154	Coelenterazine hcp	250 µg	399.48	433	445	500	2-5
21155	Coelenterazine n	250 µg	457.52	431	468	0.15	6-30
21156	Coelenterazine	1 mg	423.46	429	466	1	6-30
21157	Coelenterazine cp	1 mg	415.48	430	442	28	2-5

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21158	Coelenterazine f	1 mg	425.45	437	472	20	6-30
21159	Coelenterazine h	1 mg	407.46	437	466	16	6-30
21160	Coelenterazine hcp	1 mg	399.48	433	445	500	2-5
21161	Coelenterazine n	1 mg	457.52	431	468	0.15	6-30
21165	Coelenterazine h	10 mg	407.46	437	466	16	6-30
21166	Coelenterazine	10 mg	423.46	429	466	1	6-30
36305	Screen Quest™ Luminometric Calcium Assay Kit *10 Plates*	10 plates	N/A	N/A	N/A	N/A	N/A
36306	Screen Quest™ Luminometric Calcium Assay Kit *100 Plates*	100 plates	N/A	N/A	N/A	N/A	N/A

[3]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 using an ionophore to manipulate Ca^{2+} levels inside cells 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
20999	NAADP	1 mg	855.31
21000	NAADP, AM	1 mg	1032.64
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*	10 x 72 mg	285.36
20061	Probenecid *Water-soluble*	10 x 77 mg	307.34

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