

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
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	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
21033	Indo-1, AM *Bulk packaging*	50 mg	1009.91	346	475
21036	Indo-1, AM *Custom packaging*	20×50 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

¹Note: 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 Ca²⁺ and exhibits a quantum yield at saturating Ca²⁺ of ~0.14 and a K_d for Ca²⁺ of 390 nM.

The long-wavelength Rhod-2 is a valuable Ca²⁺ 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 Ca²⁺ than any other BAPTA-based indicator (K_d = ~320 µM) and is suitable for Ca²⁺ 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 Ca²⁺. 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) 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
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
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

21090	Quest Fluo-8H™, AM	1 mg	~1100	494	517
21091	Quest Fluo-8H™, AM	10 x 50 µg	~1100	494	517
21095	Quest Fluo-8H™, sodium salt	10 x 50 µg	~800	494	517
21096	Quest Fluo-8L™, AM	1 mg	~1100	494	517
21097	Quest Fluo-8L™, AM	10 x 50 µg	~1100	494	517
21098	Quest Fluo-8L™, sodium salt	10 x 50 µg	~800	494	517
21080	Quest Fluo-8™, AM	1 mg	~1000	494	517
21081	Quest Fluo-8™, AM	5 x 50 µg	~1000	494	517
21082	Quest Fluo-8™, AM	10 x 50 µg	~1000	494	517
21083	Quest Fluo-8™, AM	20 x 50 µg	~1000	494	517
21088	Quest Fluo-8™, sodium salt	10 x 50 µg	~800	494	517
21120	Quest Rhod-4™, AM	1 mg	~1000	524	551
21121	Quest Rhod-4™, AM	5 x 50 µg	~1000	524	551
21122	Quest Rhod-4™, AM	10 x 50 µg	~1000	524	551
21123	Quest Rhod-4™, AM	20 x 50 µg	~1000	524	551
21128	Quest Rhod-4™, sodium salt	5 x 50 µg	~800	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
36336	Cal-520™ Medium Removal Calcium Assay Kit *Optimized for Difficult Cell Lines*	1 kit	N/A	490	525
36337	Cal-520™ Medium Removal Calcium Assay Kit *Optimized for Difficult Cell Lines*	1 kit	N/A	490	525
36338	Cal-520™ No-Wash Calcium Assay Kit *Optimized for Difficult Cell Lines*	1 kit	N/A	490	525
36339	Cal-520™ No-Wash Calcium Assay Kit *Optimized for Difficult Cell Lines*	1 kit	N/A	490	525
36400	Cal-520™ PBX Calcium Assay Kit *Optimized for Probenecid-Sensitive Cell Lines*	1 kit	N/A	490	525
36401	Cal-520™ PBX Calcium Assay Kit *Optimized for Probenecid-Sensitive Cell Lines*	1 kit	N/A	490	525
36314	Screen Quest™ Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium* *1 Plate*	1 kit	N/A	490	525
36315	Screen Quest™ Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium* *10 Plates*	1 kit	N/A	490	525
36316	Screen Quest™ Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium* *10×10 Plates*	1 kit	N/A	490	525
36307	Screen Quest™ Fluo-8 NW Calcium Assay Kit *Medium Removal* *1 Plate*	1 kit	N/A	490	525
36308	Screen Quest™ Fluo-8 NW Calcium Assay Kit *Medium Removal* *10 Plates*	1 kit	N/A	490	525
36309	Screen Quest™ Fluo-8 NW Calcium Assay Kit *Medium Removal* *10×10 Plates*	1 kit	N/A	490	525
36333	Screen Quest™ Rhod-4 NW Calcium Assay Kit	1 kit	N/A	530	590

	*1% FBS Growth Medium * *1 plate*				
36334	Screen Quest™ Rhod-4 NW Calcium Assay Kit *1% FBS Growth Medium* *10 plates*	1 kit	N/A	530	590
36335	Screen Quest™ Rhod-4 NW Calcium Assay Kit *1% FBS Growth Medium* *10×10 plates*	1 kit	N/A	530	590
36330	Screen Quest™ Rhod-4 NW Calcium Assay Kit *Medium Removal* *1 plate*	1 kit	N/A	530	590
36331	Screen Quest™ Rhod-4 NW Calcium Assay Kit *Medium Removal* *10 plate*	1 kit	N/A	530	590
36332	Screen Quest™ Rhod-4 NW Calcium Assay Kit *Medium Removal* *10×10 plate*	1 kit	N/A	530	590

²Note: 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 $\sim 0.1 \mu\text{M}$ 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.

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 *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

³Notes: 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
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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