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# Fluorescent Ion and pH Indicators

## And Related Products



Biotium

# OVERVIEW

## Calcium Indicators

To study the role of the important intracellular messenger calcium in regulating various cellular events, it is essential to quantitatively monitor its concentration. The most widely used method of $Ca^{2+}$ detection is by the use of fluorescent $Ca^{2+}$ indicators, a technique pioneered by professor Roger Tsien and colleagues (1). Calcium indicators are fluorophores coupled to a BAPTA calcium chelator structure. They differ in their $Ca^{2+}$ dissociation constants ($K_d$) or $Ca^{2+}$ response range, excitation/emission wavelengths, spectral shift, and relative fluorescent quantum yields. Therefore, you should select a $Ca^{2+}$ indicator that best suits your needs in consideration of your biological system, instrument settings and any other fluorescent probes that you may use at the same time. The $K_d$ values can give you an estimate of the detectable $Ca^{2+}$ concentration range, usually 0.1 $K_d$ to 10 $K_d$. However, one should be cautious in using these *in vitro* determined $K_d$ values as the values in cells could differ considerably due to differences in ionic strength, pH, viscosity and $Ca^{2+}$ buffering by cellular lipids and proteins (2).

## Other Ion and pH Indicators

Mag-fura-2 (Table 1) can be used as a magnesium indicator or low affinity calcium indicator. We also provide a selection of fluorescent indicators for chloride and zinc (page 5), as well as pH indicators (pages 6-7).

## Cell Membrane-Permeable AM Esters

A wide selection of ion indicators are available in both the membrane-impermeant salt forms and the membrane-permeant AM ester forms. The salt forms of the indicators are water-soluble and can be loaded into cells via microinjection. The AM esters of the indicators themselves do not bind ions. However, once they have entered cells, they are readily hydrolyzed by intracellular esterases into the parent ion indicators. AM esters are membrane-permeant and thus can be loaded into cells by simple incubation of the cell or tissue preparation in a buffer containing the AM ester. Biotium also supplies Pluronic F-127, a mild non-ionic detergent that can facilitate cell loading of AM ester compounds (see page 7).

## Accessory Products

Biotium also offers calcium ionophores (page 5), as well as calibration buffers, anhydrous solvents and loading agents for AM esters, and other accessory products for ion indicator studies (page 7).

References: 1) Methods in Cell Biology, Vol. 30, 1989. pp. 127-156. 2) Cell Calcium, 1997. 21, 233.

**Table 1. Physical properties of calcium indicators**

| Indicator | MW [1] | Excitation [2] | Emission [2] | $K_d$ [3] | Page |
|---|---|---|---|---|---|
| Bis-fura-2 [4] | 779 | 363/335 nm | 512/505 nm | 370 nM | 3 |
| Fluo-3 | 770 | 506 nm | 525 nm | 390 nM | 2 |
| Fluo-4 | 737 | 494 nm | 506 nm | 335 nM | 2 |
| Fura-2 | 642 | 363/335 nm | 512/505 nm | 145 nM | 3 |
| Indo-1 | 650 | 349/331 nm | 482/398 nm | 230 nM | 3 |
| Furaptra (mag-fura-2) | 435 | 369/330 nm | 511 nm | 1.9 mM ($Mg^{2+}$) 25 uM ($Ca^{2+}$) | 3 |
| Rhod-2 | 755 | 556 nm | 576 nm | 1 uM | 4 |
| Rhod-590 | 912 | 595 nm | 616 nm | 610 nM | 4 |

[1] Molecular weights for free acid form of indicators are listed.
[2] For non-ratiometric indicators, excitation/emission with calcium is shown; for ratiometric indicators, a pair of excitation/emission wavelengths for no calcium/high calcium are shown.
[3] Calcium dissociation constant measured at 22 °C in pH 7.2 buffer.
[4] Bis-fura-2 has similar calcium response as fura-2 but with a 75% larger extinction coefficient.







## Fluo-3

Fluo-3 absorbs at 506 nm and emits at 526 nm when bound to calcium (Figure 1). It is one of the most widely used fluorescent indicators because it can be optimally excited by the commonly-used 488 nm laser line. Fluo-3 is a non-ratiometric indicator; it is essentially non-fluorescent without $Ca^{2+}$ present, but the fluorescence increases at least 40 times on $Ca^{2+}$ binding. Because fluo-3 binds $Ca^{2+}$ more weakly (higher $K_d$) than fura-2 and indo-1, it is more useful for measuring high transient $Ca^{2+}$ concentration during $Ca^{2+}$ spikes.

References: 1) J. Biol. Chem. 1989. 264, 8171. 2) J. Biol. Chem. 1989. 264, 8179.

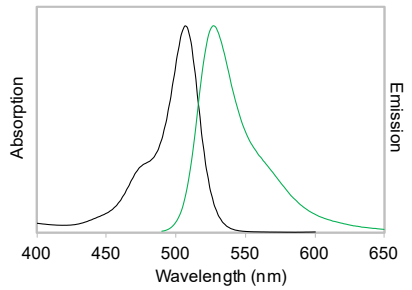
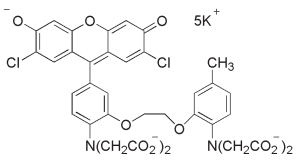
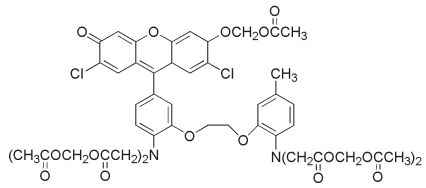



Figure 1. Absorption and emission spectra of fluo-3 saturated with $Ca^{2+}$.




Fluo-3, $5K^+$

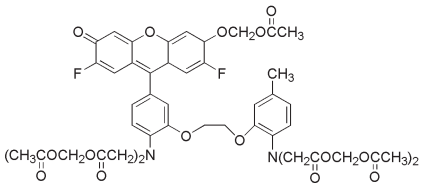



Fluo-3, AM ester

## Fluo-4

Fluo-4 is an analog of fluo-3 with the two chlorine substituents replaced by fluorines, which results in increased fluorescence excitation at 488 nm that gives higher fluorescence signal.

Reference: Cell Calcium. 2000. 27 (2), 97.




Fluo-4, AM ester

**Table 2. Fluo-3 and Fluo-4 products**

| Cat. # | Product | Unit Size |
|---|---|---|
| 50010 | Fluo-3, $5NH_4^+$ | 1 mg |
| 50011 | Fluo-3, $5K^+$ | 1 mg |
| 50012 | Fluo-3, $5Na^+$ | 1 mg |
| 50013 | Fluo-3, AM ester | 10 x 100 ug |
| 50014 | Fluo-3, AM ester | 1 mg |
| 50015 | Fluo-3, AM ester, 1 mM in anhydrous DMSO | 1 mL |
| 50016 | Fluo-3, AM ester | 20 x 50 ug |
| 50018 | Fluo-4, AM ester | 10 x 50 ug |

## Fura-2

Fura-2 is a widely used UV-excitable fluorescent calcium indicator (1-5). Upon calcium binding, the fluorescent excitation maximum of the indicator undergoes a blue shift from 363 nm ($Ca^{2+}$-free) to 335 nm ($Ca^{2+}$-saturated), while the fluorescence emission maximum is relatively unchanged at ~510 nm (Figure 2). The indicator is typically excited at 340 nm and 380 nm and the ratio of the fluorescence emission intensities is used to calculate the $Ca^{2+}$ concentration. Measurement of calcium concentration using ratiometric methods avoids artifacts due to uneven dye distribution and photobleaching (6). Fura-2 has been used in many cellular systems and applications, particularly in microscopic imaging.

Bis-fura-2 has two fura-type fluorophores coupled to one BAPTA chelator. It has a slightly lower affinity for calcium and is membrane impermeable (7-11).

References: 1) J. Mol. Cell. Cardiol. 1992. 24, 937. 2) J. Biol. Chem. 1991. 266, 23739. 3) J. Cell Biol. 1990. 110, 1555. 4) Cell Calcium. 1990. 11, 385. 5) J. Biol. Chem. 1985. 260, 3340. 6) Methods in Cell Biology, Vol. 30. 1989. p. 157. 7) J Neurophysiol. 2000. 84, 2777. 8) Brain Res. 1999. 831, 113. 9) J Neurophysiol. 1999. 81, 2508. 10) J Neurosci Res. 1999. 57, 906. 11) J. Neurochem. 1999. Int 34, 391.

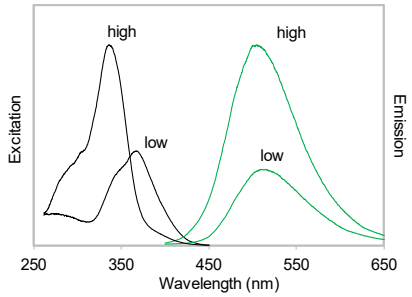
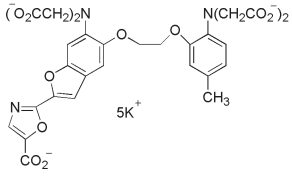
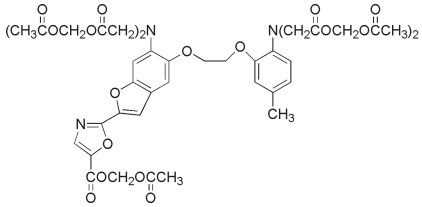



Figure 2. Absorption and emission spectra of fura-2 at high and low calcium concentrations.




Fura-2, $5K^+$

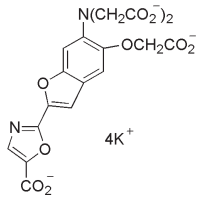


Fura-2, AM ester

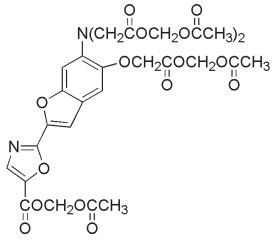
## Furaptra

Furaptra, which is also commonly called mag-fura-2, was originally developed as a fluorescent magnesium indicator, with a Kd of 1.9 mM for magnesium. However, it also is an excellent indicator for high calcium concentrations in the range of 1 uM to 100 uM (1-2). Fura-2 and furaptra share the same chromophore and as a result have similar spectral change in response to calcium concentration change.

References: 1) Pflügers Arch. 1995. 429, 587. 2) Neuron. 1993. 10, 21.




Furaptra, $4K^+$



Furaptra, AM ester



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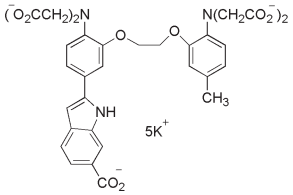
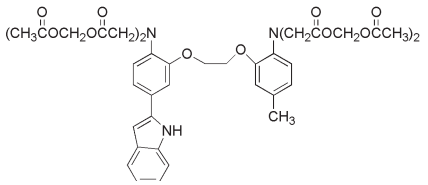
## Indo-1

Indo-1 is a UV-excitable fluorescent $Ca^{2+}$ indicator that can be optimally excited by the 351-356 nm spectral lines of the argon-ion laser. The emission maximum of the indicator shifts from ~475 nm in $Ca^{2+}$-free medium to ~400 nm in $Ca^{2+}$-saturated medium (Figure 7). $Ca^{2+}$ concentration can thus be determined by measuring the ratio of the fluorescence emission intensities at the two wavelengths. As with fura-2, this ratiometric technique avoids problems associated with uneven dye distribution and photobleaching. Indo-1 has been widely used in flow cytometry studies.

References: 1) J. Biol. Chem. 1985. 260, 3340. 2) Circulation Res. 1991. 69, 46. 3) Am. Heart J. 1990. 120, 590. 4) Cell Calcium. 1990. 11, 487. 5) Meth. Cell Biol. 1994. 41, 150.




Indo-1, $5K^+$



Indo-1, AM ester

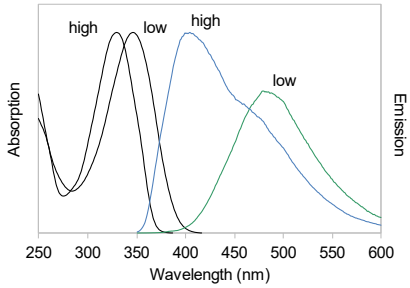



Figure 3. Absorption and emission spectra of indo-1 at high and low calcium concentrations.

**Table 3. Fura-2, Furaptra, and Indo-1 products**

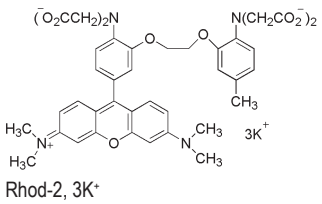
| Cat. # | Product | Unit Size |
|---|---|---|
| 50030 | Fura-2, $5NH_4^+$ | 1 mg |
| 50031 | Fura-2, $5K^+$ | 1 mg |
| 50032 | Fura-2, $5Na^+$ | 1 mg |
| 50033 | Fura-2, AM ester | 10 x 100 ug |
| 50033-1 | Fura-2, AM ester | 20 x 50 ug |
| 50034 | Fura-2, AM ester | 1 mg |
| 50029 | Fura-2, AM ester, 1 mM in anhydrous DMSO | 1 mL |
| 50045 | Bis-Fura-2, $6K^+$ | 1 mg |
| 50035 | Furaptra, $4K^+$ | 1 mg |
| 50036 | Furaptra, $4Na^+$ | 1 mg |
| 50037 | Furaptra, AM ester | 10 x 100 ug |
| 50038 | Furaptra, AM ester | 1 mg |
| 50039 | Furaptra, AM ester | 20 x 50 ug |
| 50040 | Indo-1, $5NH_4^+$ | 1 mg |
| 50041 | Indo-1, $5K^+$ | 1 mg |
| 50042 | Indo-1, $5Na^+$ | 1 mg |
| 50043 | Indo-1, AM ester | 10 x 100 ug |
| 50043-1 | Indo-1, AM ester | 20 x 50 ug |
| 50044 | Indo-1, AM ester | 1 mg |

# CALCIUM INDICATORS

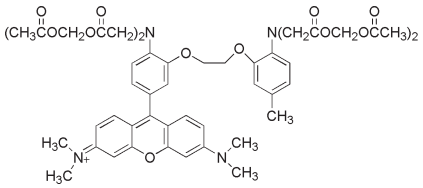
## Rhod-2

Rhod-2 is a fluorescent calcium chelator bearing a rhodamine-like fluorophore. Similar to fluo-3, rhod-2 has excitation and emission spectra that do not undergo significant shift with changing calcium concentration (Figure 4). The indicator is essentially non-fluorescent before $Ca^{2+}$ binding but becomes more fluorescent with increasing $Ca^{2+}$ concentration. Compared to Fluo-3, Rhod-2 is much less fluorescent and has lower fluorescence enhancement with $Ca^{2+}$.

References: 1) J. Physiol. 1998. 507, 4051. 2) J. Biol. Chem. 1989. 264, 8179.



Rhod-2, $3K^+$



Rhod-2, AM ester

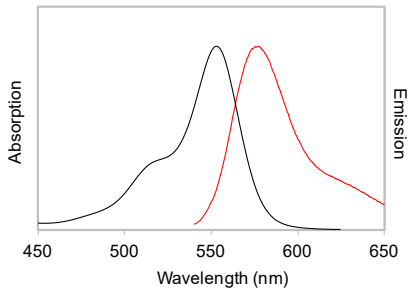
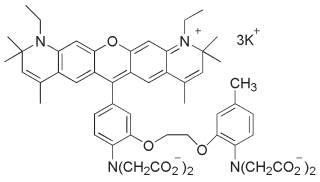


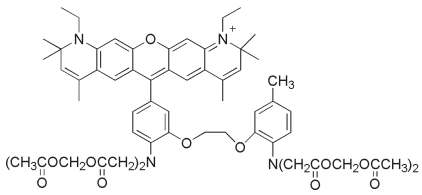

Figure 4. Absorption and emission spectra of rhod-2 saturated with $Ca^{2+}$.

## Rhod-590

Rhod-590 has absorption (595 nm) and emission (616 nm) maxima that are longer than those of fluo-3. Compared to fluo-3, rhod-590 has a smaller fluorescent enhancement with Ca2+.



Rhod-590, $3K^+$



Rhod-590, AM ester

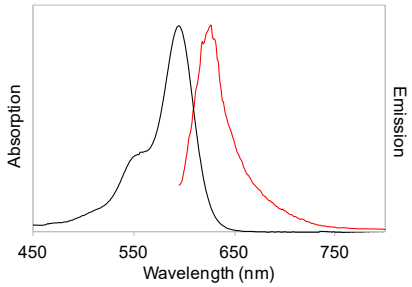



Figure 5. Absorption and emission spectra of rhod-590 saturated with $Ca^{2+}$.

**Table 4. Indo-1, Rhod-2, and Rhod-590 products**

| Cat. # | Product | Unit Size |
|---|---|---|
| 50020 | Rhod-2, $3NH_4^+$ | 1 mg |
| 50021 | Rhod-2, $3K^+$ | 1 mg |
| 50022 | Rhod-2, $3Na^+$ | 1 mg |
| 50023 | Rhod-2, AM ester | 10 x 100 ug |
| 50024 | Rhod-2, AM ester | 1 mg |
| 50026 | Rhod-590, $3K^+$ | 500 ug |
| 50025 | Rhod-590, AM ester | 10 x 50 ug |

# CALCIUM CHELATORS

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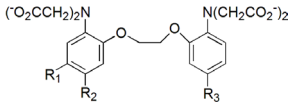
## BAPTA Chelators

BAPTA and its derivatives are calcium chelators that are commonly used to form calcium buffers with well-defined calcium concentrations. By injecting the chelators into cells or by incubating cells with the AM ester form of the chelators, one can control the cytosolic calcium concentration, an important means to study the roles of calcium.

Key advantages of these calcium chelators include relative insensitivity toward intracellular pH change and fast release of calcium. Biotium offers several BAPTA chelators with calcium dissociation constants covering the biologically significant range from $10^{-7}$ to $10^{-2}$ M.



BAPTA chelators (see table 5)

**Table 5. Physical properties of BAPTA chelators**

| Chelator | $R_1$ | $R_2$ | $R_3$ | MW * | $K_d$ (mM) ** |
|---|---|---|---|---|---|
| BAPTA | H | H | H | 477 | No $Mg^{2+}$: 0.59<br>1 mM $Mg^{2+}$: 0.70 |
| 5,5'-Dibromo BAPTA | H | Br | Br | 635 | No $Mg^{2+}$: 3.6 |
| 5,5'-Difluoro BAPTA | H | F | F | 513 | No $Mg^{2+}$: 0.61<br>1 mM $Mg^{2+}$: 0.72 |
| 5,5'-Dimethyl BAPTA | H | $CH_3$ | $CH_3$ | 505 | No $Mg^{2+}$: 0.16<br>1 mM $Mg^{2+}$: 0.44 |
| 5-Methyl-5'-nitro BAPTA | H | $NO_2$ | $CH_3$ | 536 | No $Mg^{2+}$: 53 |
| 5-Mononitro BAPTA | H | $NO_2$ | H | 522 | No $Mg^{2+}$: 94 |
| 4-Trifluoromethyl BAPTA | $CF_3$ | H | H | 545 | No $Mg^{2+}$: 0.57 |

*Molecular weights for free acid forms are listed
**Cell Calcium 10, 491 (1989).

**Table 6. List of BAPTA products**

| Cat. # | Product | Unit Size |
|---|---|---|
| 50001 | BAPTA, $4Cs^+$ | 1 g |
| 50002 | BAPTA, $4K^+$ | 1 g |
| 50003 | BAPTA, $4Na^+$ | 1 g |
| 50000 | BAPTA, AM ester | 25 mg |
| 50000-1 | BAPTA, AM ester | 20 x 1 mg |
| 50004 | 5,5'-Dibromo BAPTA, $4K^+$ | 100 mg |
| 50005 | 5,5'-Difluoro BAPTA, AM ester | 25 mg |
| 50006 | 5,5'-Difluoro BAPTA, $4K^+$ | 100 mg |
| 50007 | 5,5'-Dimethyl BAPTA, AM ester | 25 mg |
| 50008 | 5,5'-Dimethyl BAPTA, $4K^+$ | 100 mg |
| 50009 | 5-methyl-5'-nitro BAPTA, $4K^+$ | 10 mg |
| 50017 | 5-mononitro BAPTA, $4K^+$ | 10 mg |

# IONOPHORES

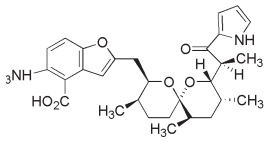
## Calcium ionophores

Calcium ionophores are ion carriers that facilitate the equilibration of calcium across cellular membranes. They are useful tools for calibrating calcium indicators and manipulating intracellular calcium concentration. Biotium offers the ionophores listed below.

### A-23187

A-23187 (calcimycin, calcium ionophore III) is a calcium ionophore that rapidly equilibrates intracellular and extracellular calcium concentrations. It is commonly used for in situ calibration of fluorescent calcium indicators. The ionophore allows $Mn^{2+}$ to enter the cells and quench intracellular fluorescence of calcium indicators.

References: 1) Anal. Chem. 1989. 61, 382. 2) FASEB J. 1994. 8, 639. 3) Merck Index 12, 1678. 4) Science. 1982. 217, 943.

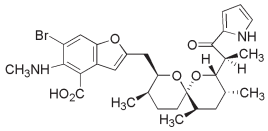


A-23187, free acid

### 4-Bromo-A23187

The calcium ionophore 4-bromo A-23187 is similar to A-23187. However, because 4-bromo A-23187 is non-fluorescent, it is preferred over the blue fluorescent A-23187 for calibrating UV-excited $Ca^{2+}$ indicators such as indo-1 and fura-2 in order to keep background fluorescence to a minimum.

Reference: Anal. Chem. 1985. 146, 349.

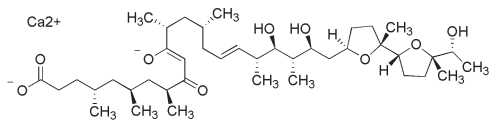


4-Bromo A-23187, free acid

### Ionomycin

Ionomycin is commonly used to modify intracellular calcium concentrations and to calibrate fluorescent calcium indicators. Ionomycin is commonly used to stimulate cytokine production and cell proliferation in lymphocytes. In other cell types it can induce apoptosis and inhibit proliferation.

References: 1) J. Biol. Chem. 2000. 275, 7071. 2) J. Immunol. 1995. 155, 3297. 3) J. Urol. 1999. 162, 916.



Ionomycin, calcium salt

**Table 7. Calcium ionophore products**

| Cat. # | Product | Unit Size |
|---|---|---|
| 59001 | A-23187, free acid | 1 mg |
| 59006 | 4-Bromo A-23187, free acid | 1 mg |
| 59007 | Ionomycin, calcium salt | 1 mg |

# OTHER ION INDICATORS

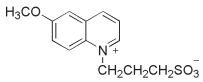


## Chloride indicators

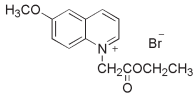
The fluorescence of SPQ 6(-methoxy-N-(3-sulfopropyl)quinolinium) is specifically quenched by chloride via collision. Therefore, chloride concentration is measured by monitoring the degree of fluorescence decrease. The dye can be loaded into cells by hypotonic shock. The dye has excitation/emission at 344/443 nm (1-3).

MQAE (N-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide) is an improved chloride indicator that has greater sensitivity to chloride ($K_{sv}$ = 200 $M^{-1}$) than SPQ ($K_{sv}$ = 118 $M^{-1}$) and higher fluorescence quantum yield. The ester group of MQAE may slowly hydrolyze inside cells, resulting in a change in its fluorescence response. The dye has excitation/emission at 350/460 nm (4-6).

References: 1) Neurosci. Lett. 1989. 104, 326. 2) Biophys. J. 1989. 56, 1071. 3) J. Biol. Chem. 1991. 266, 20590. 4) Am J Physiol. 1990. 259, C375. 5) Anal Biochem. 1989. 178, 355. 6) Anal Biochem. 1996. 241, 51.



SPQ

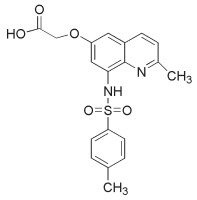


MQAE

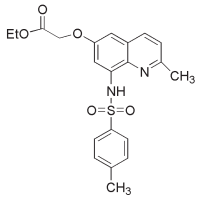
## Zinc indicators

Zinc is believed to be involved in the suppression of apoptosis and play important roles in many neural activities. Zinquin is an UV-excitable, blue fluorescent zinc indicator with excitation/emission at 350/460 nm. Zinquin free acid is membrane-impermeant, while Zinquin ethyl ester is membrane-permeable and is hydrolyzed into Zinquin free acid after entering cells.

References: 1) Biochem. J. 1994. 303, 781. 2) Biochem. J. 1993. 296, 403.



Zinquin free acid



Zinquin ethyl ester

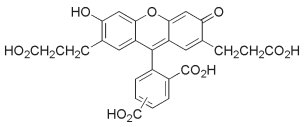
**Table 8. Other ion indicators**

| Cat. # | Product | Unit Size |
|---|---|---|
| 52010 | SPQ 6(-methoxy-N-(3-sulfopropyl)quinolinium) | 50 mg |
| 52011 | MQAE (N-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide) | 100 mg |
| 52022 | Zinquin free acid | 5 mg |
| 52020 | Zinquin ethyl ester | 5 mg |

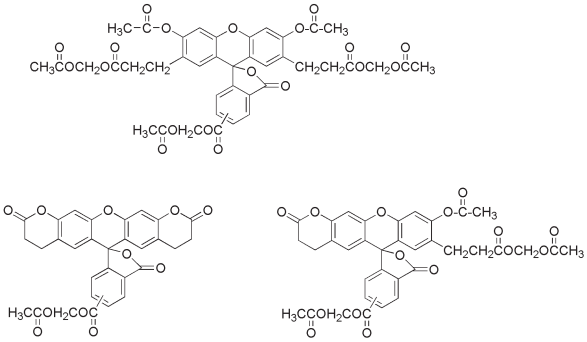
## BCECF

BCECF is the most widely used fluorescent pH sensor. With a pKa of 6.97, close to physiological pH, BCECF can detect changes in cytosolic pH with high sensitivity. At low pH, the dye is weakly fluorescent but becomes more fluorescent with increasing pH. The excitation spectrum of the dye undergoes a slight shift with pH change, while the wavelength of the emission maximum remains unchanged. The pH is determined ratiometrically by the relative fluorescent intensities at 535 nm when the dye is excited at 439 nm and 505 nm. Available in membrane-impermeant free acid and cell-permeable AM ester forms.

References: 1) J. Immunol. Meth. 1988. 108, 255. 2) Biochem. J. 1994. 304, 751. 3) J. Immunol. Meth. 1994. 172, 255.



BCECF



BCECF AM ester (mixture of 3 compounds)

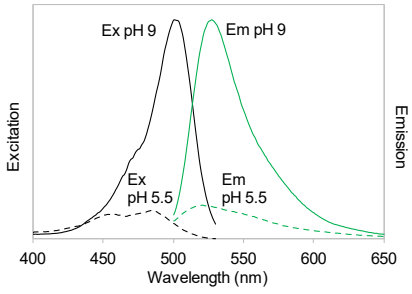



Figure 6. Excitation and emission spectra of BCECF at pH 5.5 and pH 9.0

## Carboxyfluorescein derivatives

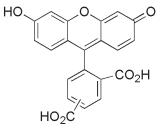
Carboxyfluorescein (FAM) has a $pK_a$ of 6.5 and can be used as a pH indicator or cellular tracer. Its excitation spectrum and fluorescence response to pH are similar to those of BCECF (1). Carboxyfluorescein is also available in membrane permeable diacetate form (CFDA) (2-5). CFDA-SE is a membrane-permeable amine-reactive form that covalently reacts with cytoplasmic proteins for stable labeling (6-8).

5-(and-6)-Carboxy-2',7'-dichlorofluorescein is similar to carboxyfluorescein, but has a pKa of 4.8, and therefore is useful for detecting pH in more acidic environment (9-11). It is also available in membrane-permeable diacetate, and membrane-permeable, amine-reactive diacetate succinimidyl ester forms.

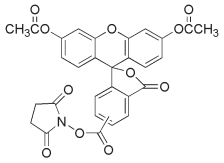
The pKa of 5-carboxy-2',7'-dichlorosulfonefluorescein is near 4.0 and thus the dye is potentially an excellent pH indicator for acidic organelles.

Fluorescein derivatives are available as single isomer or mixed isomers; the isomers have the same spectral properties. For certain coupling reactions, single isomer may be preferable, but mixed isomers are suitable for most applications.

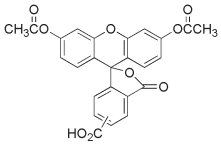
References: 1) Methods Enzymol. 1986. 128, 65. 2) J. Cell Sci. 1991. 98, 343. 3) Nature. 1982. 295, 524. 4) J. Immunol. Meth. 1987. 100, 261. 5) J. Immunol. Meth. 1980. 33, 33. 6) J. Cell Biol. 1985. 101, 610. 7) J. Cell Biol. 1986. 103, 2649. 8) Cell Transplantation. 1994. 3, 397. 9) Biochemistry. 1987. 26, 6330. 10) Biochim. Biophys. Acta. 1989. 985, 75. 11) Anal. Biochem. 1990. 187, 109.



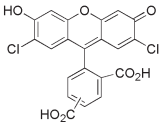
5(6)-FAM



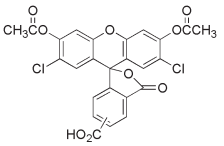
5(6)-CFDA-SE



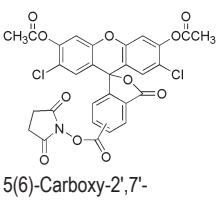
5(6)-CFDA



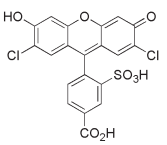
5-(and-6)-Carboxy-2',7'-dichlorofluorescein



5-(and-6)-Carboxy-2',7'-dichlorofluorescein diacetate



5(6)-Carboxy-2',7'-dichlorofluorescein diacetate, SE.



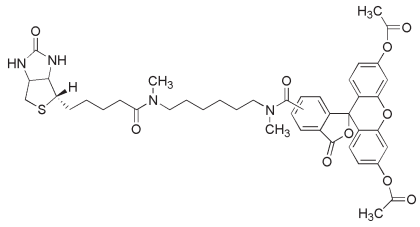
5-Carboxy-2',7'-dichlorosulfonefluorescein

# pH INDICATORS

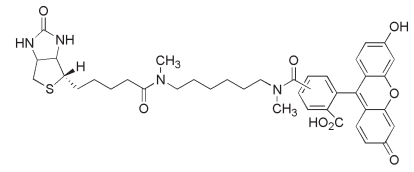
## Flubida-2 and Flubi-2

Flubida-2 has been used to detect pH at a specific site in a cell such as cell organelles by directing the probe to where avidin-chimera proteins are located. The probe is a conjugate of biotin and fluorescein diacetate, which is non-fluorescent until the probe has entered the cells and is hydrolyzed by cellular esterases. Flubida-2 is membrane-permeable and can be delivered into cells via simple incubation. Flubi-2 is the membrane-impermeable hydrolyzed product of Flubida-2. Its excitation spectrum and fluorescence response to pH are similar to those of BCECF.

Reference: Chemistry & Biology. 2000. 7, 197.



Flubida-2



Flubi-2

**Table 9. pH Indicators**

| Cat. # | Product | Unit Size |
|---|---|---|
| 51010 | BCECF, free acid | 1 mg |
| 51011 | BCECF, AM ester | 10 x 100 ug |
| 51011-1 | BCECF, AM ester | 20 x 50 ug |
| 51012 | BCECF, AM ester | 1 mg |
| 51009 | BCECF, AM ester, 1 mg/mL in anhydrous DMSO | 1 mL |
| 51019 | 5-Carboxyfluorescein (5-FAM, single isomer) | 100 mg |
| 51020 | 6-Carboxyfluorescein (6-FAM, single isomer) | 100 mg |
| 51013 | 5-(and-6)-Carboxyfluorescein (5(6)-FAM, mixed isomers) | 100 mg |
| 51018 | 5-CFDA (5-Carboxyfluorescein diacetate, single isomer) | 100 mg |
| 51021 | 6-CFDA (6-Carboxyfluorescein diacetate, single isomer) | 100 mg |
| 51014 | 5(6)-CFDA (5-(and-6)-Carboxyfluorescein diacetate, mixed isomers) | 100 mg |
| 90041 | 5(6)-CFDA, SE (5-(and-6)-Carboxyfluorescein diacetate, succinimidyl ester) | 25 mg |
| 51017 | 5-Carboxy-2',7'-dichlorofluorescein | 100 mg |
| 51015 | 5-(and-6)-Carboxy-2',7'-dichlorofluorescein | 200 mg |
| 51016 | 5-(and-6)-Carboxy-2',7'-dichlorofluorescein diacetate | 100 mg |
| 90040 | 5-(and-6)-Carboxy-2',7'-dichlorofluorescein diacetate, succinimidyl ester (SE) | 25 mg |
| 51023 | 5-Carboxy-2',7'-dichlorosulfonefluorescein | 10 mg |
| 51024 | Flubi-2 | 5 mg |
| 51022 | Flubida-2 | 5 mg |

# ACCESSORY REAGENTS

Distributed by: CliniSciences Group

## Calcium Calibration Kit

The Calcium Calibration Kit is used to prepare buffers with a range of accurate calcium concentrations, and is useful for the calibration of fluorescent $Ca^{2+}$ indicators (1-2). The kit contains 50 mL each of zero calcium buffer and 40 uM free $Ca^{2+}$ buffer, with a detailed protocol for combining the two buffers to obtain a $Ca^{2+}$ calibration curve from 0.017-39.8 uM free calcium.

Component A (zero free $Ca^{2+}$):
Zero mM CaEGTA (10 mM $K_2$EGTA, 100 mM KCl and 10 mM MOPS; pH 7.20)

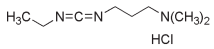
Component B (40 uM free $Ca^{2+}$):
10 mM CaEGTA (10 mM CaEGTA, 100 mM KCl and 10 mM MOPS; pH 7.20)

References: 1) Cell Calcium. 1991. 12, 279. 2) Neuropharmacology. 1995. 34, 1423.

## EDC for fixation of indicators

EDC (or EDAC) has recently been found to be very useful for fixing chelators in situ, including the fluorescent ion indicators in this catalog. The fixation of ion indicators makes it useful for post histological studies following the physiological experiments. EDC is also a widely used reagent to activate carboxy groups for amine coupling.

References: 1) Cell Calcium. 1997. 21(3), 175. 2) Tetrahedron Lett. 1993. 34, 7685.

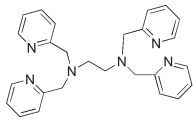


EDC (EDAC)

## TPEN heavy metal chelator

TPEN is an excellent heavy metal chelator, but it does not affect $Ca^{++}$, $Mg^{++}$, $Na^{+}$ or $K^{+}$ concentrations. Thus, TPEN is a useful tool to identify effects of heavy metals ($Zn^{++}$, $Fe^{++}/Fe^{+++}$, $Cu^{++}$, and $Mn^{++}$, etc.) on fluorescent indicators of $Ca^{++}$, $Mg^{++}$, $Na^{+}$ and $K^{+}$.

Reference: J. Biol. Chem. 1985. 260, 2719.



TPEN

## Pluronic F-127 for AM ester loading

Pluronic F-127 is a non-ionic detergent that is useful for facilitating loading of AM ester compounds into cells.

References: 1) Science. 1986. 233, 886. 2) J. Biol. Chem. 1987. 262, 12801.

## Anhydrous DMSO

Anhydrous Dimethylsulfoxide (DMSO) is recommended for preparing stock solutions of moisture-sensitive AM ester compounds.

**Table 10. Accessory products**

| Cat. # | Product | Unit Size |
|---|---|---|
| 59100 | Calcium Calibration Kit | 1 kit |
| 59002 | EDC (EDAC) | 100 mg |
| 59003 | TPEN (tetrakis-(2-pyridylmethyl)ethylenediamine) | 100 mg |
| 59000 | Pluronic F-127 | 1 g |
| 59004 | Pluronic F-127, 20% in DMSO | 1 mL |
| 59005 | Pluronic F-127, 10% in H2O | 30 mL |
| 90082 | DMSO, anhydrous | 10 mL |

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