

Physical properties of fluorescent calcium indicators



Calcium is an important intracellular messenger ion that regulates numerous cellular processes. To study the role of calcium in cells, 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 (Tsien 1989).

Biotium offers a number of Ca^{2+} indicators, including fura-2, indo-1, fluo-3 and rhod-2. The primary differences among indicators are 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 need 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 $0.1K_d$ to $10K_d$. However, one should be cautious in using these in vitro determined K_d values as the K_d values in cells could differ considerably due to differences in ionic strength, pH, viscosity and Ca^{2+} buffering by intracellular lipids and proteins (Petr and Wurster 1997).

Calcium indicators are available in both membrane-impermeant salt forms and 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 Ca^{2+} . However, once they enter cells, they are readily hydrolyzed by intracellular esterases into the parent Ca^{2+} indicators, thus becoming responsive to Ca^{2+} . Calcium indicator AM esters are membrane-permeant and can be loaded into cells by simple incubation of the cell or tissue preparation in a buffer containing the AM ester. Biotium supplies Pluronic F-127, a mild non-ionic detergent that can facilitate cell loading of AM ester compounds. Anhydrous DMSO, also available from Biotium, is recommended for preparing stock solutions of AM esters to prevent hydrolysis of the AM ester group during storage and handling.

Biotium also offers related products for calcium studies, including calcium chelators, calcium ionophores, caged calcium, and calcium calibration buffers.

References

Tsien, R. *Methods in Cell Biology* 30, Taylor, D.L. and Wang, Y-L, Eds., Academic Press (1989), pp. 127-156

Petr, M.J. and Wurster, R.D. *Cell Calcium*, 21, 233 (1997)

Table 1. Physical properties of calcium indicators

Indicator Name	Mwt. ¹	Detection Mode ²	K_d (nM) ³
Bis-fura-2 ⁴	779	Ex: 340/380 nm	370
Fluo-3	770	Em: 525 nm	390
Fura-2	642	Ex: 340/380 nm	145
Indo-1	650	Em: 405/485 nm	230
Mag-fura-2	435	Ex: 340/380 nm	25,000
Rhod-2	755	Ex: 578 nm	1,000

¹ Molecular weights for free acid form of indicators are listed.

² Indicators for which a pair of wavelengths are listed can be used for ratiometric calcium measurements.

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.