BTC Ion Indicators

Catalog nos. B6790, B6791

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
BTC, tetrapotassium salt (Cat. no. B6790)	1 mg	• ≤–20˚C • Desiccate • Protect from light	When stored as directed this product is stable for at least 6 months.
BTC, acetoxymethyl (AM) ester (Cat. no. B6791)	100 µg		
Approximate fluorescence excitation/emission maxima: See Table 2.			

Introduction

The benzothiazole-based indicator BTC (Figure 1) is designed for dual-excitation ratio measurements of Ca^{2+} and Zn^{2+} concentrations, respectively. BTC was developed by Invitrogen in collaboration with Haralambos Katerinopoulos at the University of Crete.[1,2] The relatively low Ca^{2+}-binding affinity of BTC (K_d = 7 µM) is suitable for detecting elevated calcium levels associated with activation of smooth muscle, neurons, and intracellular calcium stores, as well as for kinetic tracking of Ca^{2+} transients.[2,3] BTC, unlike fura-2, is capable of distinguishing Ca^{2+} levels in the micromolar range produced by neurotoxic glutamate receptor activation from submicromolar levels associated with non-lethal excitotoxicity (Figure 2).[4]

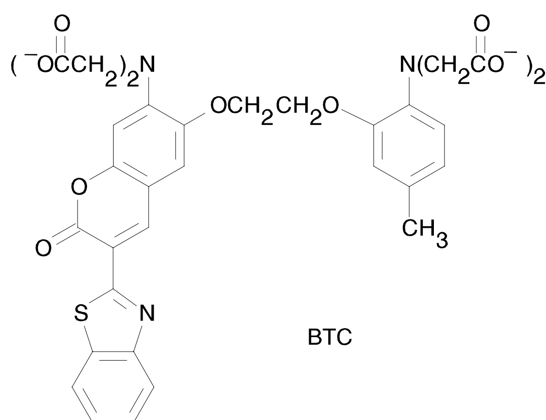

Figure 1. Structure of BTC Ca^{2+} indicator.

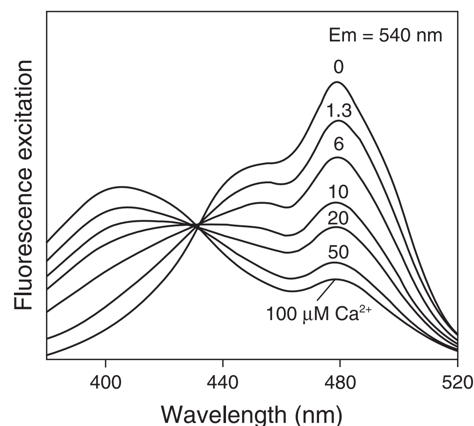

Figure 2. Fluorescence excitation spectra of BTC (B6790) in solutions containing 0–100 µM free Ca^{2+}.

Table 2. Properties of the BTC indicator.

Indicator	Ion-free		Ion-bound			K_d (Ion)*
	Ex/Em**	ε_{max} (cm^{-1} M^{-1}) †	Ex/Em**	ε_{max} (cm^{-1} M^{-1}) †	QY‡	
BTC	464/533	29,000	401/529	20,000	0.12	7.0 µM (Ca^{2+})§

*K_d values depend on pH, temperature, ionic strength, and other factors, and are usually significantly higher in cellular environments. **Ex/Em: Fluorescence excitation and emission maxima in nm. †Molar extinction coefficient at absorption maximum. ‡QY: fluorescence quantum yield. § K_d determined at 22°C in 100 mM KCl, 10 mM MOPS pH 7.2, K_d = 15.2 µM in the presence of 1 mM Mg^{2+}.

Properties

BTC exhibits fluorescence excitation shift on binding Ca^{2+} and Zn^{2+}. A summary of physical and spectroscopic properties is shown in Table 2.

Before Starting

Preparing Stock Solutions and Storage

Prepare the stock solutions of the solid tetrapotassium BTC salt in distilled water or aqueous buffers. Store solutions frozen (≤–20°C) and **protected from light**. When stored properly, these solutions are stable for at least six months.

Store BTC AM esters desiccated and **protected from light** at ≤–20°C; AM esters are susceptible to hydrolysis (particularly in solution) but can be stored for at least six months in the vials as received. Reconstitute AM esters in anhydrous dimethylsulfoxide (DMSO) and store frozen (≤–20°C), desiccated, and **in the dark.** Avoid repeated freezing and thawing.

To check for possible AM ester degradation, perform the following simple test in a fluorometer. Dilute a small aliquot of AM ester stock solution to a final concentration of about 1 µM in calcium-free buffer. Transfer the solution to a cuvette and measure the fluorescence intensity using appropriate wavelength settings (Table 2). Add calcium to a saturating concentration (≥100 µM) and check fluorescence again. There should be no significant difference in measured fluorescence between the two readings. Significant changes in fluorescence upon calcium addition (*i.e.*, in the second reading) indicate partial hydrolysis of the AM ester.

Experimental Protocols

Cell Loading Guidelines

You may load the water-soluble salt form of the BTC indicator into cells by microinjection, patch pipette infusion,[4] or using our Influx™ pinocytotic cell-loading reagent (Cat. no. I14402). The following loading protocols using cell-permeant AM esters are provided as an introductory guide only; refer to published procedures for details.[6]

1.1 Remove the AM ester stock solution in DMSO from the freezer and allow to warm to room temperature before opening.

1.2 Dilute an aliquot of DMSO stock solution (1–5 mM) to a final concentration of 1–5 µM in the buffered physiological medium of choice.

Addition of non-ionic detergent Pluronic® F-127 can assist in dispersing the non-polar AM ester in aqueous media. You can conveniently accomplish this by mixing the aliquot of AM ester stock solution in DMSO with an equal volume of 20% (w/v) Pluronic in DMSO (Cat. no. P3000MP) before diluting in loading medium, making the final Pluronic concentration about 0.02%. Invitrogen offers Pluronic® F-127 in 30 mL units of a sterile 10% (w/v) solution in water (Cat. no. P6866) and 2 g solid units (Cat. no. P6867).

1.3 You may add the organic anion transport inhibitors probenecid (1–2.5 mM) or sulfinpyrazone (0.1–0.25 mM) to the cell medium to reduce leakage of the de-esterified indicator.[7] Stock solutions of sulfinpyrazone and probenecid are necessarily quite alkaline; it is therefore important that you readjust the pH of media to which you add them.

1.4. Incubate the cells with the AM ester for 15 to 60 minutes at 20°C to 37°C.

Note: You do not need to lift adherent cultures for loading.

You need to empirically determine the exact loading concentration, time, and temperature; in general it is desirable to use the minimum dye concentration required to yield fluorescence signals with adequate signal to noise levels. Subcellular compartmentalization, an inherent problem with the AM ester loading technique, is usually lessened by lowering the incubation temperature.

1.5 Before you begin fluorescence measurements, wash cells in indicator-free medium (containing anion transport inhibitor, if applicable) to remove any dye that is nonspecifically associated with the cell surface, and then incubate for a further 30 minutes to allow complete de-esterification of intracellular AM esters.

Response Calibration

You can calibrate indicators such as BTC that show an excitation spectral shift upon ion binding using a ratio of the fluorescence intensities measured at two different wavelengths, which cancels artifactual variations in the fluorescence signal that might otherwise be misinterpreted as changes in ion concentration. Ratiometric measurements reduce or eliminate variations of several determining factors in the measured fluorescence intensity, including indicator concentration, excitation pathlength, excitation intensity, and detection efficiency.[8,9] Artifacts that are eliminated include photobleaching and leakage of the indicator, variable cell thickness, and nonuniform indicator distribution within cells (due to compartmentalization) or among populations of cells (due to loading efficacy variations). The following equation is used to determine the effective dissociation constant of the indicator (K_d) from measurements of fluorescence intensity ratios (R) in solutions with precisely known free Ca^{2+} concentrations. Calibration solutions based on EGTA Ca^{2+} buffering[10,11] are supplied in a variety of convenient formats in Molecular Probes' Calcium Calibration Buffer Kits. When K_d is known, the same equation is used to obtain $[Ca^{2+}]_{free}$ for experimental samples from measured values of R.

$$\left[Ca^{2+}\right]_{free} = K_d\, Q \frac{(R - R_{min})}{(R_{max} - R)}$$

where R represents the fluorescence intensity ratio $F_{\lambda 1}/F_{\lambda 2}$, in which $\lambda 1$ and $\lambda 2$ are the fluorescence excitation wavelengths for the ion-bound and ion-free indicator, respectively. Ratios corresponding to the titration end points are denoted by the subscripts indicating the <u>min</u>imum and <u>max</u>imum Ca^{2+} concentration. Q is the ratio of F_{min} to F_{max} at $\lambda 2$. Note that you must subtract background levels from the component fluorescence intensities before calculating the ratios. A suitable $\lambda 1/\lambda 2$ combination for BTC is 400/480 nm. It is important to recognize that the calcium-binding and spectroscopic properties of fluorescent indicators can vary quite markedly in cellular environments.[12] You can control perturbations to calcium measurements caused by the presence of heavy metal cations such as Zn^{2+}, Mn^{2+}, and Cd^{2+} by using the selective chelator TPEN (Cat. no. T1210). *In situ* response

calibrations of intracellular indicators typically yield K_d values significantly higher than *in vitro* determinations.[13] Perform *in situ* calibrations by exposing loaded cells to controlled Ca^{2+} buffers in the presence of ionophores such as A-23187 (Cat. no. A1493), 4-bromo A-23187 (Cat. no. B1494), or ionomycin.[14] It is assumed that all the intracellular indicator is de-esterified and available for ion binding.

References

1. Cell Calcium 15, 190 (1994); **2.** Cell Calcium 27, 75 (2000); **3.** Biophys J 68, 2156 (1995); **4.** J Neuroscience 17, 6669 (1997); **5.** Neuron 26, 187 (2000); **6.** *Cell Biology: A Laboratory Handbook, 2nd Edition*, J.E. Celis, Ed., Volume 3, pp 363–374, Academic Press (1998); **7.** Cell Calcium 11, 57 (1990); **8.** Methods Cell Biol 30, 157 (1989); **9.** J Biol Chem 260, 3440 (1985); **10.** Methods Cell Biol 40, 3 (1994); **11.** Methods Enzymol 172, 230 (1989); **12.** Cell Calcium 28, 225 (2000); **13.** Biophys J 70, 896 (1996); **14.** Cell Calcium 21, 233 (1997).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
B6790	BTC, tetrapotassium salt *cell impermeant*	1 mg
B6791	BTC, AM *cell permeant*	100 µg
I14402	Influx™ pinocytic cell-loading reagent *makes 10 x 5 mL*	1 set
P10020	PowerLoad™ concentrate, 100X	5 mL
P3000MP	Pluronic® F-127 *20% solution in DMSO*	1 mL
P6866	Pluronic® F-127 *10% solution in water* *0.2 µm filtered*	30 mL
P6867	Pluronic® F-127 *low UV absorbance*	2 g
T1210	tetrakis-(2-pyridylmethyl)ethylenediamine (TPEN)	100 mg

Contact Information

Molecular Probes, Inc.
29851 Willow Creek Road
Eugene, OR 97402
Phone: (541) 465-8300
Fax: (541) 335-0504

Customer Service:
6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338
Fax: (541) 335-0305
probesorder@invitrogen.com

Toll-Free Ordering for USA:
Order Phone: (800) 438-2209
Order Fax: (800) 438-0228

Technical Service:
8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353
Toll-Free (800) 438-2209
Fax: (541) 335-0238
probestech@invitrogen.com

Invitrogen European Headquarters
Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

For country-specific contact information, visit www.invitrogen.com.

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Limited Use Label License No. 223: Labeling and Detection Technology
The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402, Tel: (541) 465-8300. Fax: (541) 335-0354.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.

Copyright 2009, Molecular Probes, Inc. All rights reserved. This information is subject to change without notice.