

Fluorescent Indicators for Chloride

M-440	SPQ, 6-methoxy-<i>N</i>-(3-sulfopropyl)quinolinium, inner salt
E-3101	MQAE, <i>N</i>-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide
L-6868	lucigenin (bis-<i>N</i>-methylacridinium nitrate) *high purity*
M-6886	MEQ, 6-methoxy-<i>N</i>-ethylquinolinium chloride

Introduction

Measurement of intracellular Cl⁻ concentrations and the study of Cl⁻ channels have been stimulated by the discovery that the genetic effects of cystic fibrosis are manifested by changes in Cl⁻ transport.¹ However, most current techniques for investigating the kinetics of Cl⁻ transport in cells are seriously limited. Methods based on measurement of ³⁶Cl have poor sensitivity because of the low specific activity of this isotope, and microelectrodes have poor Cl⁻ selectivity and can be used only in large cells. Fluorescent indicators are proving to be more sensitive and selective than conventional methods and in some cases can be loaded into cells noninvasively. We offer these fluorescent Cl⁻ indicators:

- 6-methoxy-*N*-(3-sulfopropyl)quinolinium (SPQ, M-440)
- *N*-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide (MQAE, E-3101)
- 6-methoxy-*N*-ethylquinolinium iodide (MEQ, M-6886)
- lucigenin (L-6868)

Some of the properties of Molecular Probes' chloride indicators are summarized in Table 1. All of the above dyes are collisionally quenched by halide ions,^{2,3} resulting in an ion concentration-dependent fluorescence decrease without a shift in wavelength (Figure 1). The efficiency of the quenching process is represented by the Stern-Volmer quenching constant (K_{SV}), which is the reciprocal of the ion concentration that produces 50% of maximum quenching. Quenching by other halides, such as Br⁻ and I⁻, and other anions, such as thiocyanate and nitrite, is more efficient than Cl⁻ quenching. Fortunately, physiological concentrations of nonchloride ions do not significantly affect the fluorescence of SPQ and analogous Cl⁻ indicators. Fluorescence of SPQ has been reported to be pH-sensitive in certain buffers.⁴ Because the Cl⁻-dependent quenching of these indicators may be different in cells than in solution, intracellular indicators should be calibrated using high-K⁺ buffers and the K⁺/H⁺ ionophore nigericin (N-1495) in conjunction with tributyltin chloride, an organometallic compound that acts as a Cl⁻/OH⁻ antiporter.⁵⁻⁷

Methods for measuring Cl⁻ transport in vesicles, liposomes and live cells, including a discussion on SPQ and MQAE, have been reviewed by Verkman.⁵ Ultraviolet-excited indicators (SPQ, MQAE and MEQ) can be excited using the argon-ion laser lines at 351 nm and 364 nm for confocal microscopy⁸ and flow cytometry⁹ applications.

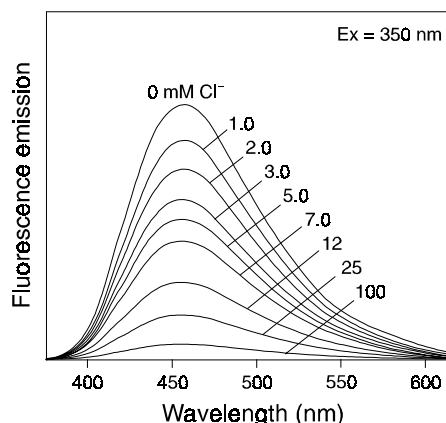


Figure 1. Fluorescence emission spectra of MQAE in increasing concentrations of Cl⁻.

SPQ

SPQ (M-440) is usually loaded into cells by transient hypotonic shock permeabilization. For example, SPQ-loaded lymphocytes were prepared for flow cytometry⁹ by incubation of cells ($8-10 \times 10^6$ /mL) in hypotonic medium for 15 minutes at 37°C. The hypotonic medium comprised Hanks' balanced salt solution (HBSS) diluted 1:1 with water, and contained 5 mM SPQ. At the end of the loading period, 100 μ L samples of cell suspension were diluted 15:1 in HBSS, centrifuged, resuspended in 200 μ L of HBSS and then incubated for a further 15 minutes at 37°C to allow recovery from the hypotonic shock. This procedure produced minimal leakage of indicator — the fluorescence intensity of loaded cells decreased by less than 10% in the 15-minute period immediately after completion of loading. Viability of loaded cells was >95% as assessed by exclusion of propidium iodide.

Chloride permeability assays, used to detect activity of the cystic fibrosis transmembrane conductance regulator (CFTR) and other anion transporters^{1,10,11} use SPQ-loaded cells that are successively perfused with chloride-containing extracellular medium followed by medium in which the chloride content is replaced by nitrate (NO₃⁻). These assays take advantage of the fact that NO₃⁻ produces no quenching of SPQ fluorescence, although its channel permeability is essentially the same as Cl⁻.¹⁰ Quenching of SPQ fluorescence by Cl⁻ is less efficient inside cells ($K_{SV} = 12 \text{ M}^{-1}$) than in aqueous solution ($K_{SV} = 118 \text{ M}^{-1}$).¹²

Table 1. Properties of Molecular Probes' chloride indicators.

Cat #	Acronym	MW	Abs (nm) *	ϵ (cm ⁻¹ M ⁻¹)	Em (nm) *	K _{sv} (M ⁻¹) †
E-3101	MQAE	326	350	2800	460	200
L-6868	lucigenin	511	455	7400	505	390
M-440	SPQ	281	344	3700	443	118
M-6886	MEQ	315	344	3900	442	145

* All spectra are in water. All these products undergo Cl⁻-dependent fluorescence quenching with essentially no change in absorption or emission wavelengths.
† Values of K_{sv} are taken from literature sources: Anal Biochem 219, 139 (1994); Biochemistry 30, 7879 (1991); Anal Biochem 178, 355 (1989).

MQAE

Developed by Verkman and colleagues, the improved Cl⁻ indicator MQAE (E-3101) has greater sensitivity to Cl⁻ (K_{sv} = 200 M⁻¹) than SPQ (K_{sv} = 118 M⁻¹)^{5,6} and a higher fluorescence quantum yield. In cells, K_{sv} values for MQAE of 25–28 M⁻¹ have been determined in various cell types.^{13,14} In a study of Cl⁻ transport in liposomes and LLC-PK1 cells, MQAE was found to undergo slow leakage from cells (<20% per hour at 37°C).⁶ Its fluorescence was unaffected by pH or by bicarbonate, borate, nitrate, or sulfate anions,⁶ though some quenching by H₂PO₄⁻ and carboxylates has been reported.¹³ The ester group of MQAE may slowly hydrolyze inside cells, resulting in a change in its fluorescence response.¹³

MEQ and Cell-Permeant diH-MEQ

The Cl⁻ indicator 6-methoxy-*N*-ethylquinolinium iodide (MEQ) can be rendered cell-permeant by masking its positively charged nitrogen to create a lipophilic, Cl⁻-insensitive compound, 6-methoxy-*N*-ethyl-1,2-dihydroquinoline (diH-MEQ).^{15,16} This reduced quinoline derivative can then be loaded non-invasively into cells, where it is rapidly reoxidized to the cell-impermeant, Cl⁻-sensitive MEQ. Because diH-MEQ is susceptible to spontaneous oxidation upon storage, we offer the diH-MEQ precursor MEQ (M-6886), along with a simple protocol for reducing MEQ to the cell-permeant derivative with sodium borohydride (not supplied) just prior to cell loading. Using this protocol, researchers may find that MEQ provides an improvement over poorly retained SPQ and MQAE for investigating intracellular Cl⁻ levels. Quenching of MEQ fluorescence by Cl⁻ has a K_{sv} of 19 M⁻¹ in cells, a value that is slightly higher than that reported for SPQ in fibroblasts.

Lucigenin

The fluorescence of lucigenin (L-6868) is quantitatively quenched by Cl⁻ with a reported K_{sv} = 390 M⁻¹.¹⁷ Lucigenin absorbs maximally at both 368 nm (ϵ = 36,000 cm⁻¹M⁻¹) and 455 nm (ϵ = 7400 cm⁻¹M⁻¹), with an emission maximum at 505 nm. Its fluorescence emission has a quantum yield of ~0.6 and is insensitive to nitrate, phosphate and sulfate. Lucigenin is a useful Cl⁻ indicator in liposomes and reconstituted membrane vesicles; however, it is reportedly unsuitable for determining intracellular Cl⁻.¹⁷ Lucigenin is also a potentially valuable tool for measuring Cl⁻ efflux from cells in which the extracellular medium is suddenly made Cl⁻-free by superfusion. Lucigenin from Molecular Probes has been highly purified to remove a bright

blue fluorescent contaminant that is found in some commercial samples and can interfere with these measurements.

Storage and Handling

SPQ, MQAE, MEQ, and lucigenin are packaged as solids. Store desiccated and protected from light until ready for use. Stock solutions of SPQ, MQAE, and SPQ may be made in water or buffers to at least 1 mg/mL. Aqueous stock solutions should be stored at -20°C, protected from light.

Intracellular Chloride Determination

The following is a synopsis of the method used to calculate [Cl_i] using fluorescent chloride indicators. The relationship between fluorescence intensity of the chloride indicator and chloride concentration is given by the Stern–Volmer equation:

$$(F_0/F) - 1 = K_{sv}[Q]$$

where F₀ is the fluorescence intensity without halide or other quenching ions; F is the fluorescence intensity in the presence of quencher; [Q] is the concentration of quencher; and K_{sv} is the Stern–Volmer constant.

It is desirable to calibrate the system to obtain the K_{sv} of the indicator in the cell type being studied, using linear regression analysis according to the Stern–Volmer equation. The Stern–Volmer constant for chloride can be significantly reduced in cells due to collisional quenching by anionic species such as peptides. To fix chloride concentrations in the cell for calibration purposes, one generally clamps the levels using tributyltin and nigericin (N-1495) at concentrations between 10 and 30 μM. The fluorescence will decrease with increasing halide concentration. To maintain osmolarity it is desirable to replace chloride with an anion that does not quench the dye. Gluconate has a K_{sv} of approximately 7 M⁻¹, and so it may be used to replace Cl⁻ quite effectively.

Tributyltin chloride is an organometallic compound that acts as a chloride–hydroxide antiporter. Chloride ion concentrations equilibrate across the plasma membrane of cells kept in high potassium buffers containing tributyltin chloride and the K⁺–H⁺ ionophore nigericin (N-1495).^{5,9,12} These reagents allow the *in situ* calibration of fluorescent chloride indicators.

References

1. Proc Natl Acad Sci USA 88, 7500 (1991); 2. Fresenius Z Anal Chem 314, 577 (1983); 3. J Heterocyclic Chem 19, 841 (1982); 4. Am J Physiol 264, C27 (1993); 5. Am J Physiol 259, C375 (1990); 6. Anal Biochem 178, 355 (1989); 7. Biophys J 56, 1071 (1989); 8. J Neurosci Methods 75, 127 (1997); 9. Cytometry 28, 316 (1997); 10. Biochim Biophys Acta 1152, 83 (1993); 11. Am J Physiol 274, C310 (1998); 12. Biophys J 53, 955 (1988); 13. Anal Biochem 241, 51 (1996); 14. Am J Physiol 267, H2114 (1994); 15. Br J Pharmacol 108, 469 (1993); 16. Biochemistry 30, 7879 (1991); 17. Anal Biochem 219, 139 (1994).

Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
E-3101	<i>N</i> -(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide (MQAE)	100 mg
L-6868	lucigenin (bis- <i>N</i> -methylacridinium nitrate) *high purity*	10 mg
M-6886	6-methoxy- <i>N</i> -ethylquinolinium iodide (MEQ)	100 mg
M-440	6-methoxy- <i>N</i> -(3-sulfopropyl)quinolinium, inner salt (SPQ)	100 mg
N-1495	nigericin, free acid	10 mg

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