SBFI and PBFI

S-1262 SBFI, tetraammonium salt *cell impermeant*
S-1263 SBFI, AM *cell permeant*
S-1264 SBFI, AM *special packaging*
P-1265 PBFI, tetraammonium salt *cell impermeant*
P-1267 PBFI, AM *special packaging*

Introduction

The sodium-sensitive dye, SBFI, and the potassium-sensitive dye, PBFI, are selective ion indicators for the fluorometric determination of Na^+ and K^+ concentrations, respectively. These benzofuran isophthalate derivatives and their cell-permeant acetoxymethyl (AM) esters provide spatial and temporal resolution of Na^+ or K^+ concentrations with sufficient selectivity in the presence of physiological concentrations of other monovalent cations.[1] Furthermore, their spectral responses upon ligand binding permit excitation ratio measurements with the same filters and instrumentation as used for fura-2.[2]

These sodium and potassium indicators consist of fluorophores linked to the nitrogens of a crown ether with a cavity size that confers selectivity for the respective ligand (Figure 1). In the absence of Na^+ or K^+ at pH 7, the extinction coefficient for SBFI and PBFI is approximately 42,000 $cm^{-1}M^{-1}$ at 346 nm. Upon ion binding, the excitation peaks narrow significantly and the excitation maxima shift to shorter wavelengths, causing a large change in the ratio of energy absorbed at 340 nm/380 nm (Figure 2). Binding of Na^+ to SBFI induces a 2.5-fold enhancement of fluorescence intensity with little change in emission maximum. The fluorescence is relatively unaffected by changes in pH between 6.5 and 7.5, though it is strongly affected by ionic strength and increases with viscosity.[1,2]

Although these ion indicators are quite selective for sodium and potassium, there is some effect of K^+ on the Na^+ affinity of SBFI and of Na^+ on the K^+ affinity of PBFI. The K_d of SBFI for Na^+ is 11.3 mM in the presence of physiological concentrations of K^+ and 3.8 mM without K^+. SBFI is ~18-fold more selective for Na^+ than for K^+ (Figure 2).

Likewise, the K^+ dissociation constant of PBFI is strongly dependent on whether Na^+ is present. The K_d of PBFI for K^+ varies from about 100 mM to 10 mM in the presence and absence (respectively) of Na^+.[1] Although PBFI is only 1.5-fold more selective for K^+ than for Na^+, this is often sufficient since there is normally about 10 times more K^+ than Na^+ in cells. When used intracellularly, the K_d of these indicators should always be calibrated with the appropriate ionophore (see *Application*).

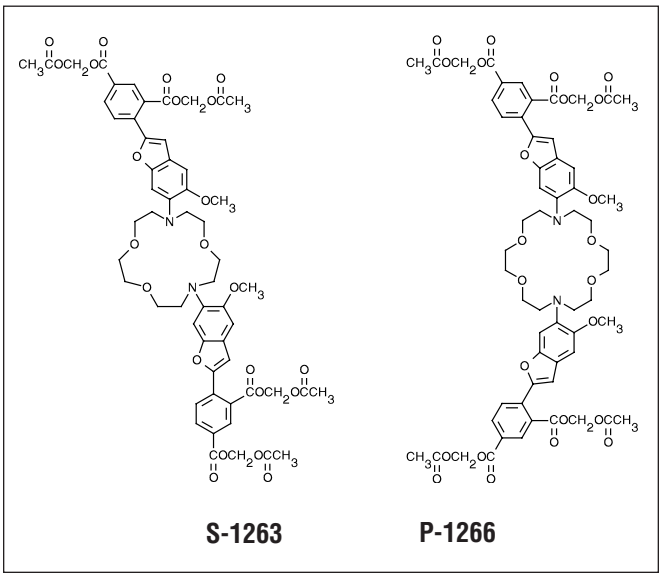

Figure 1. Chemical structures of the AM esters of sodium indicator SBFI (S-1263) and potassium indicator PBFI (P-1266).

Storage and Handling

These products are provided as lyophilized solids and should be stored desiccated and protected from light until use; the acid salts may be stored at room temperature, 4°C or –20°C without compromising stability, whereas the AM esters should be stored at –20°C. Allow products to warm to room temperature before opening. The tetraammonium salts of SBFI and PBFI may be reconstituted in aqueous buffers or distilled water; store aqueous stock solutions at –20°C and protected from light. The AM esters are susceptible to hydrolysis, particularly in solution. They should be reconstituted just before use in high-quality, anhydrous DMSO. We offer the AM esters of SBFI and PBFI packaged in 20 separate vials (S-1264, P-1267), each containing 50 µg for reconstitution in DMSO as required. DMSO stock solutions of the AM esters may be stored desiccated at –20°C and protected from light for up to six months, provided they are not subjected to freeze-thaw cycles.

Application

A generally suitable stock solution of the AM esters of SBFI and PBFI may be prepared by dissolving the material in anhydrous DMSO to a concentration of 10 mM. The molecular weights of SBFI AM and PBFI AM are 1127 and 1171, respectively. The use of Pluronic® F-127 is essential for optimal cell loading of both SBFI AM and PBFI AM due to the poor aqueous solubility of the dyes.[1,3] For the convenience of our customers, Molecular Probes offers Pluronic F-127 in three forms: 1 mL of a 20% (w/v) solution in DMSO (P-3000), 30 mL of a 0.2 µm–filtered 10% (w/v) solution in water (P-6866) and 2 g solid (P-6867). The indicator stock solution in DMSO is typically mixed with an equal volume of 25% w/v Pluronic F-127 solution immediately prior to its addition to the cell loading buffer. Loading concentrations range from 5 µM to 10 µM and loading times vary between 40 minutes and 4 hours. Explicit cell loading protocols for a variety of cell types and vesicle preparations can be obtained from the literature.[2-9] Water-soluble SBFI tetraammonium salt can be loaded into cells by diffusion from a patch pipette for correlated fluorescence imaging and electrophysiological recording.[10] Because the K_d of the indicator may be different in cells than in solution, intracellular SBFI should be calibrated using the pore-forming antibiotic gramicidin (G-6888), and intracellular PBFI using the K^+ ionophore valinomycin [2,9,11] (V-1644). A review by Negulescu and Machen [2] provides a detailed account of Na^+ measurements and calibrations using SBFI. Up-to-date product application bibliographies are available at our Web site (www.probes.com).

Measurements are generally made by exciting these indicators at 340 nm, where fluorescence is particularly sensitive to the ion concentration of interest, and at 380 nm, very near the isosbestic point. The ratio of the fluorescence intensities obtained by exciting SBFI or PBFI at these wavelengths (340/380 nm) while monitoring emission at 500 nm is then used to determine the concentration of the Na^+ or K^+ respectively. Dual-excitation filter sets for fluorescence microscopy applications are available from Omega Optical Inc. (www.omegafilters.com, set XF04) and Chroma Technology Corp. (www.chroma.com, set 71000).

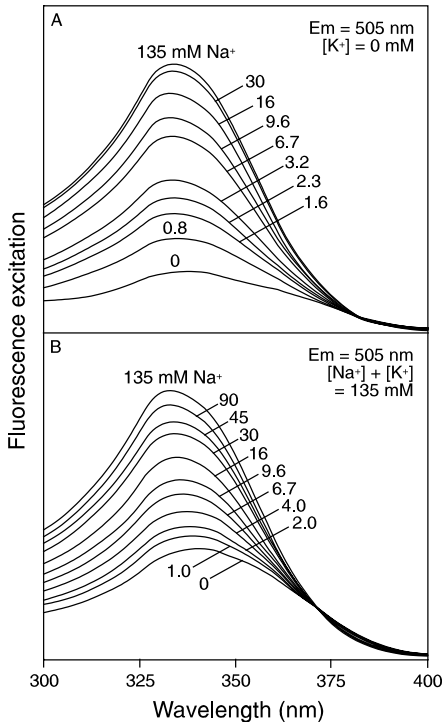

Figure 2. SBFI's excitation spectral response to Na^+: A) in K^+-free solution and B) in solutions containing K^+ with the combined Na^+ and K^+ concentration equal to 135 mM. The scale on the vertical axis is the same for both panels.

References

1. J Biol Chem 264, 19449 (1989); **2.** Methods Enzymol 192, 38 (1990); **3.** Am J Physiol 262, F462 (1992); **4.** Biochem Biophys Res Commun 164, 212 (1989); **5.** J Biol Chem 264, 19458 (1989); **6.** Am J Physiol 259, C19 (1990); **7.** J Biol Chem 265, 19543 (1990); **8.** Biochem Biophys Res Commun 175, 611 (1991); **9.** J Physiol 448, 493 (1992); **10.** J Physiol 517, 135 (1999); **11.** J Biol Chem 265, 10522 (1990).

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

Cat #	Product Name	Unit Size
P-1267	PBFI, AM *cell permeant* *special packaging*	20 x 50 µg
P-1265	PBFI, tetraammonium salt *cell impermeant*	1 mg
S-1263	SBFI, AM *cell permeant*	1 mg
S-1264	SBFI, AM *cell permeant* *special packaging*	20 x 50 µg
S-1262	SBFI, tetraammonium salt *cell impermeant*	1 mg
V-1644	valinomycin	25 mg

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