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Sodium Green™ Indicator

S-6900 Sodium Green™, tetra(tetramethylammonium) salt *cell impermeant*
S-6901 Sodium Green™ tetraacetate, *cell permeant* *special packaging*

Introduction

The sodium indicator Sodium Green™ is a visible light-excitable probe developed at Molecular Probes for the fluorometric determination of Na^+ concentration. We offer the cell-impermeant tetra(tetramethylammonium) salt of Sodium Green (S-6900), as well as the cell-permeant tetraacetate (S-6901) for monitoring intracellular levels of sodium. Like the ultraviolet-light–excitable sodium indicator SBFI, Sodium Green provides spatial and temporal resolution of Na^+ concentrations with sufficient selectivity in the presence of physiological concentrations of other monovalent cations.[1,2] In addition, the wavelengths required to excite Sodium Green allow simultaneous measurements of Na^+ and other physiological parameters, such as pH and membrane potential, and do not overlap significantly with those necessary for photoactivation of caged compounds. The spectral characteristics of this indicator have three further advantages: 1) The peak excitation and emission wavelengths are in regions of the spectrum where cellular autofluorescence [3] and scattering backgrounds are often less of a problem; 2) the energy of the excitation light is low, thus reducing the potential for photodamage of the cell; and 3) the wavelengths required for optimal excitation are compatible with those produced by laser-based instrumentation, such as confocal laserscanning microscopes.

Sodium Green consists of a fluorescein analog linked to each of the nitrogens of a crown ether with a cavity size that confers selectivity for the Na^+ ion (Figure 1). In the absence of Na^+ at pH 7, its extinction coefficient is approximately 160,000 cm^{-1} M^{-1} at 507 nm. As compared to SBFI, Sodium Green exhibits greater selectivity for Na^+ than K^+ (41-fold versus 18-fold) and displays a much higher quantum yield in Na^+-containing solutions. Upon binding to Na^+, Sodium Green exhibits an increase in fluorescence emission intensity with little shift in wavelength (Figure 2). The dissociation constant (K_d) of Sodium Green for Na^+ is about 6 mM at 22°C in potassium-free solution, and about 21 mM at 22°C in solutions containing both Na^+ and K^+ (with a total ion concentration of 135 mM), values that are similar to those of SBFI. The K_d depends upon factors such as pH, temperature, ionic strength, levels of other cations and protein concentration. When used intracellularly, the K_d of Sodium Green should always be calibrated with the appropriate ionophore (see *Response Calibration*).

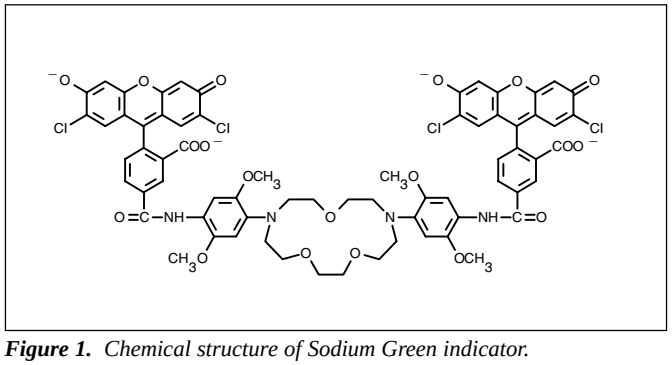

Figure 1. *Chemical structure of Sodium Green indicator.*

Storage and Handling

These products are provided as lyophilized solids. The tetra-(tetramethylammonium) salt (S-6900) may be stored protected from light at room temperature, 4°C or -20°C without compromising stability; the tetraacetate (S-6901) should be stored desiccated and protected from light at -20°C until use. Allow the products to warm to room temperature before opening. The tetra-(tetramethylammonium) salt of this sodium indicator may be reconstituted in aqueous buffers or distilled water; store aqueous stock solutions at -20°C and protected from light. When stored properly, the salt is stable for at least six months in most physiological buffers.

Sodium Green tetraacetate is quite susceptible to hydrolysis, particularly in solution, and should be reconstituted just before use in high-quality, anhydrous DMSO (available from Aldrich Chemical Co., Fluka Chemical Co. and other sources). DMSO stock solutions may be divided into aliquots and stored desiccated at -20°C, protected from light. Under these conditions, the tetraacetate should be stable for several months, providing the solvent remains anhydrous. To prevent repeated exposure to moisture, we provide Sodium Green tetraacetate packaged in sets of 20 separate vials, each containing 50 µg for reconstitution in DMSO as required. Dilute aqueous solutions of the tetraacetate for cell loading should be used on the same day that they are prepared.

Application

The cell-permeant Sodium Green tetraacetate will freely diffuse across cell membranes. Once inside the cell, intracellular esterases cleave off the acetate moieties to convert the probe into the sodium-responsive acidic form. The negatively charged groups of the acidic form greatly reduce the rate of passive leakage from the cell. We provide the following loading protocol as an introductory guide only. Loading protocols for a variety

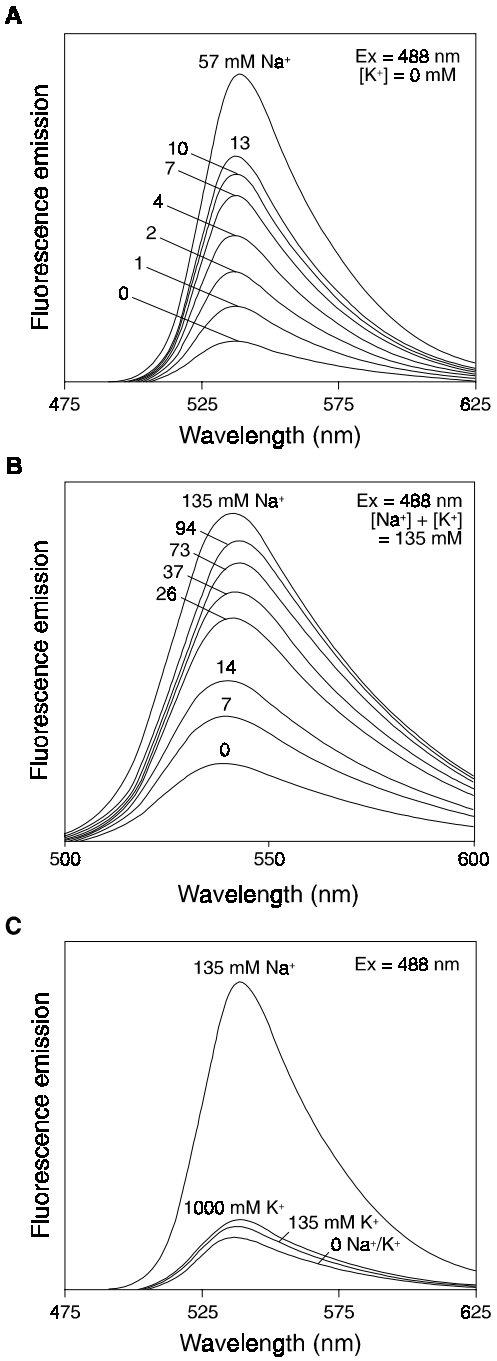

Figure 2. *Fluorescence emission response of Sodium Green A) in increasing concentrations of Na^+ and B) in solutions containing both Na^+ and K^+ where the combined concentration of the two ions equals 135 mM. In C) Sodium Green's emission response in Na^+-containing solutions is compared to its response in K^+-containing solutions. The scale on the vertical axis is different for each panel.*

of cell types with similar probes can be obtained from the literature.[4,5]

Sample Loading Protocol for Sodium Green Tetraacetate

1.1 Prepare a 2–5 mM stock solution of Sodium Green tetraacetate (molecular weight 1543) in high-quality, anhydrous DMSO. DMSO stock solutions may be divided into aliquots and stored desiccated at -20°C as described in *Storage and Handling*.

1.2 On the day of the experiment, either dissolve the lyophilized Sodium Green tetraacetate in DMSO to 2–5 mM or thaw an aliquot of the indicator stock solution to room temperature. The nonionic detergent Pluronic® F-127 (P-3000, P-6866, P-6867) is sometimes used to increase the aqueous solubility of the nonpolar tetracetate derivative. A 20% (w/v) solution of Pluronic F-127 in DMSO (P-3000) can be used in place of DMSO to prepare solutions of this sodium indicator; however, long-term storage of the tetraacetate in the presence of Pluronic F-127 is not recommended. Molecular Probes' researchers typically mix a 5 mM Sodium Green tetraacetate stock solution in DMSO with an equal volume of 20% (w/v) Pluronic F-127 in DMSO. This Sodium Green tetraacetate solution is then diluted into the loading buffer of choice to achieve a final concentration of 1–10 µM tetraacetate and less than 0.1% detergent. The exact concentration of Sodium Green tetraacetate required for cell loading must be determined empirically. To avoid sodium buffering, toxicity and other artifacts of overloading, one should generally use the lowest probe concentration that yields sufficient signal.

1.3 Incubate cells with Sodium Green tetraacetate for 20 minutes to one hour at or below room temperature. In our laboratory, NIH/3T3 cells required a loading time of just 30 minutes, whereas much longer times are often required when loading the AM ester of SBFI. Adherent cultures do not need to be lifted for loading. Some investigators report that decreasing the loading temperature reduces indicator compartmentalization.[4]

1.4 Wash cells to remove excess probe that either has not been loaded or may be noncovalently associated with the membrane. For fluorescence microscopy applications, Omega bandpass filter sets XF104 or XF23 and Chroma filter sets 41028 or 31001 are suitable for selective detection of Sodium Green. Omega® filters are supplied by Omega Optical Inc. (www.omegafilters.com). Chroma filters are supplied by Chroma Technology Corp. (www.chroma.com).

Response Calibration

The peak excitation and emission wavelengths for Sodium Green are 507 nm and 532 nm, respectively. Calibration is typically accomplished using the tetra(tetramethylammonium) salt of the indicator in solutions of precisely known free sodium concentrations. To determine either the free sodium concentration of a solution or the K_d of a single-wavelength sodium indicator, the following equation is used:

$$\left[Na^+\right]_{free} = K_d\left[\frac{F - F_{min}}{F_{max} - F}\right]$$

where F is the fluorescence of the indicator at experimental sodium levels, F_{min} is the fluorescence in the absence of sodium and F_{max} is the fluorescence of the sodium-saturated probe. The dissociation constant (K_d) is a measure of the affinity of the probe for sodium.

Because the calibration solutions may not reflect the intracellular environment, the values for F_{max} and F_{min} are most accurately ascertained using cells that have been loaded with the tetraacetate form of the indicator and then treated with a pore-forming antibiotic, gramicidin[5] (G-6888). Although free sodium concentration determined levels using single-wavelength dyes are inherently less accurate than those measured using dual-wavelength ratioable indicators such as SBFI, published comparisons show good quantitative agreement.[2]

References

1. J Biol Chem 264, 19449 (1989); **2.** Cytometry 21, 248 (1995); **3.** J Biol Chem 271, 29067 (1996); **4.** Methods Enzymol 307, 119 (1999); **5.** Methods Enzymol 192, 38 (1990).

Product List

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

Cat #	Product Name	Unit Size
G-6888	gramicidin	100 mg
P-6867	Pluronic® F-127 *low UV absorbance*	2 g
P-3000	Pluronic® F-127 *20% solution in DMSO*	1 mL
P-6866	Pluronic® F-127 *sterile 10% solution in water*	30 mL
S-6901	Sodium Green™ tetraacetate *cell permeant* *special packaging*	20x50 µg
S-6900	Sodium Green™, tetra(tetramethylammonium) salt *cell impermeant*	1 mg

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