CoroNa™ Green Sodium Indicator

CoroNa™ Green *cell impermeant* (C36675)
CoroNa™ Green, AM *cell permeant* *special packaging* (C36676)

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Desiccate
- Protect from light

Abs/Em for CoroNa Green/Na⁺ complex: 492/516 nm

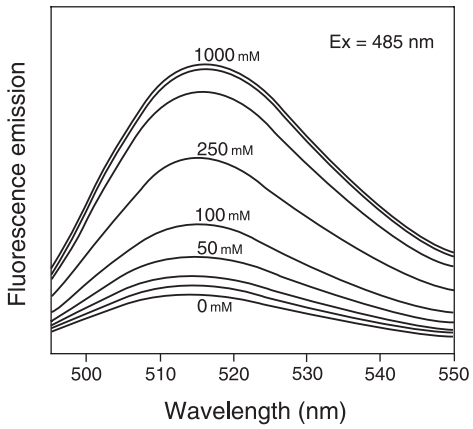

Figure 1. Fluorescence emission spectra of the CoroNa Green dye in 50 mM MOPS, pH 7.0 (adjusted with tetramethylammonium hydroxide), containing 100 mM K^+ and variable concentrations of Na^+ as indicated.

Introduction

The CoroNa™ Green dye is an improved green-fluorescent sodium (Na^+) indicator that exhibits an increase in fluorescence emission intensity upon binding Na^+, with little shift in wavelength (Figure 1). Similar to our other sodium indicators, SBFI and Sodium Green, the CoroNa Green indicator allows spatial and temporal resolution of Na^+ concentrations selectively in the presence of physiological concentrations of other monovalent cations. Comprising of a fluorescein molecule linked to a crown ether with a cavity size that confers selectivity for the Na^+ ion (Figure 2), the CoroNa Green indicator is less than half the size of the Sodium Green dye (molecular weight 586 and 1668, respectively). The smaller size appears to help the cell-permeant CoroNa Green AM dye load cells better than the Sodium Green tetraacetate dye — under similar conditions, <3% of a cell population was successfully loaded with the Sodium Green dye, compared to ~20% with the CoroNa Green dye (data not shown). Furthermore, the CoroNa Green indicator responds to a broader range of Na^+ concentration (Figure 3), with a K_d of ~80 mM. With absorbance and fluorescence emission maxima of 492 and 516 nm, respectively, for the Na^+-bound form, the CoroNa Green dye can be detected by any instrument that can detect fluorescein, including flow cytometers, microscopes, and fluorescent microplate readers and fluorometers. We expect that this reagent will play a valuable role in understanding the role of extra- and intracellular levels of sodium.

Materials

Contents

The cell-impermeant CoroNa Green indicator (C36675) is supplied in a unit size of 1 mg. The cell-permeant AM ester of CoroNa Green (C36676) is supplied as sets of 20 tubes containing 50 µg each.

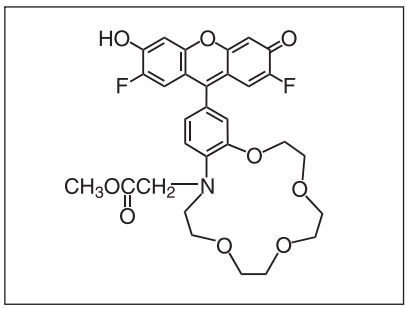

Figure 2. Structure of the CoroNa Green sodium indicator.

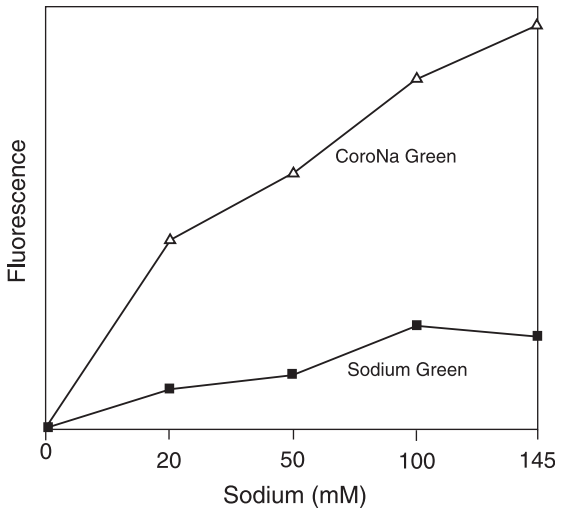

Figure 3. Flow cytometric detection of live cells loaded with the CoroNa Green AM or Sodium Green tetraacetate dye. Jurkat cells were loaded with 10 µM dye for one hour at 37°C. Cells were washed and resuspended in sodium gluconate in the presence of 20 µM monensin for 30 minutes at 37°C.

Storage and Handling

Upon receipt, store the cell-impermeant CoroNa Green indicator (C36675) and the cell-permeant acetoxymethyl (AM) ester of CoroNa Green (C36676) at ≤–20ºC, desiccated and protected from light. Allow the products to warm room temperature before opening. The cell-impermeant CoroNa Green indicator may be reconstituted in DMSO, and then diluted in aqueous buffers for use. CoroNa Green AM ester should be reconstituted just before use in high-quality, anhydrous DMSO. DMSO stock solutions may be divided into aliquots and stored desiccated at ≤–20°C and protected from light.

Application

The cell-permeant CoroNa Green AM 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 form. The following procedure is designed as a guide and may require adaptation in some cases.

Cell Loading

1.1 Suspend cells in Hanks' balanced salt solution (HBSS) or other physiological saline medium. Add the CoroNa Green indicator at 0.5–10 µM by dilution from a concentrated stock solution in DMSO.

1.2 Incubate cells for 10–45 minutes at 37°C. Wash loaded cells twice with HBSS before measuring fluorescence.

Fluorescence Detection

In aqueous solutions, the CoroNa Green indicator has absorption and fluorescence emission maxima at approximately 492 and 516 nm, respectively. Optical filter sets designed for detection of fluorescein (FITC) or Alexa Fluor® 488 dye are suitable for fluorescence microscopy applications.

Response Calibration

Response calibration can be carried out by measuring the fluorescence intensity of the CoroNa Green indicator in solutions with precisely known free Na^+ concentrations. The dissociation constant for the indicator–ion complex (K_d) may be determined using the following equation, where F denotes fluorescence intensity and the subscripts *min* and *max* identify the data points corresponding to the minimum and maximum ion concentrations, respectively.

$$[Na^+] = K_d\,(F - F_{min})/(F_{max} - F)$$

When K_d is known, the same equation can be used to obtain $[Na^+]$ values corresponding to measured fluorescence intensities (F) of experimental samples. It is important to recognize that the ion-binding and spectroscopic properties of fluorescent indicators can vary quite markedly in cellular environments. Consequently, *in situ* response calibrations of intracellular indicators often yield K_d values that are significantly different from *in vitro* determinations.[1–3] *In situ* calibrations of sodium indicators are performed by exposing loaded cells to controlled Na^+ concentrations in the presence of the ionophore gramicidin (G6888) at a working concentration of 2–10 µM.[3–5]

References

1. J Physiol 498, 295-307 (1997); **2.** Am J Physiol 276, H1581-H1590 (1999); **3.** Methods Enzymol 192, 38-81 (1990); **4.** J Biol Chem 264, 19458-19467 (1989); **5.** Cytometry 21, 248-256 (1995).

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

Cat #	Product Name	Unit Size
C36675	CoroNa™ Green *cell impermeant*	1 mg
C36676	CoroNa™ Green, AM *cell permeant* *special packaging*	20 x 50 µg
G6888	gramicidin	100 mg

Contact Information

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

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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

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