Fura-10™, postassium salt

Catalog number: 21110, 21111
Unit size: 5x50 ug, 1 mg

Component	Storage	Amount (Cat No. 21110)	Amount (Cat No. 21111)
Fura-10™, postassium salt	Freeze (< -15 °C), Minimize light exposure	5x50 ug	1 mg

OVERVIEW

In contrast to single-wavelength indicators such as Fluo-4, the absorption (or fluorescence excitation) maximum of Fura indicators shifts from 380 nm (Fura-2), 415 nm (Fura-8™ and Fura-10™) for the Ca2+-free chelator to about 340 nm (Fura-2), 355 nm (Fura-8™ and Fura-10™) for the Ca2+-bound . The wavelength of maximum fluorescence emission is relatively independent of Ca2+ concentration. The largest dynamic range for Ca2+-dependent fluorescence signals is obtained by using excitation at 340 nm and 380 nm (for Fura-2), 355 nm and 415 nm (for Fura-8™ and Fura-10™) and ratioing the fluorescence intensities detected at ~510 nm (Fura-2), 525 nm (Fura-8™ and Fura-10™). From this ratio, the level of intracellular Ca2+ can be estimated, using dissociation constants (Kd) that are derived from calibration curves. By using the ratio of fluorescence intensities produced by excitation at two wave lengths, factors such as uneven dye distribution and photo bleaching are minimized because they should affect both measurements to the same extent. Calibration solutions should be initially free of heavy metal ions such as manganese, which may affect both its fluorescence and its affinity for calcium.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Fura-10™ stock solution
Prepare a 2 to 5 mM Fura-10™ stock solution in ddH_2O.

PREPARATION OF WORKING SOLUTION

Fura-10™ working solution
Prepare a 10 to 50 µM Fura-10™ working solution in ddH_2O or buffer of your choice.

SAMPLE EXPERIMENTAL PROTOCOL

In contrast to single-wavelength indicators such as Fluo-4, the absorption (or fluorescence excitation) maximum of Fura indicators shifts from 415 nm (Fura-10™) for the Ca^{2+} -free chelator to about 355 nm (Fura-10™) for the Ca^{2+} -bound. The wavelength of maximum fluorescence emission is relatively independent of Ca^{2+} concentration. The largest dynamic range for Ca^{2+} -dependent fluorescence signals is obtained by using excitation at 355 nm and 415 nm (Fura-10™) and ratioing the fluorescence intensities detected at ~510 nm 525 nm (Fura-10™). From this ratio, the level of intracellular Ca^{2+} can be estimated, using dissociation constants (Kd) that are derived from calibration curves. By using the ratio of fluorescence intensities produced by excitation at two wave lengths, factors such as uneven dye distribution and photo bleaching are minimized because they should affect both measurements to the same extent. Calibration solutions should be initially free of heavy metal ions such as manganese, which may affect both its fluorescence and its affinity for calcium.

Once the indicator has been calibrated with solutions of known Ca^{2+} concentrations (see below), the following equation can be used to relate the intensity ratios to Ca^{2+} levels:

$[Ca^{2+}] = Kd\ Q\ (R - Rmin) / (Rmin - R)$

Where, R represents the fluorescence intensity ratio $F_{\lambda1}/F_{\lambda2}$, in which λ1 (~ 355 nm for Fura-10™) and λ2 (415 nM Fura-10™) are the fluorescence detection wavelengths for the ion-bound and ion-free indicator, respectively. Ratios corresponding to the titration end points are denoted by the subscripts indicating the minimum and maximum Ca^{2+} concentration. Q is the ratio of Fmin to Fmax at λ2 (~ 415 nM Fura-10™). Kd is the Ca^{2+} dissociation constant of the indicator. Calibrating fura indicators requires making measurements for the completely ion-free and ion-saturated indicator (to determine the values for Fmin, Fmax, Rmin, and Rmax) and for the indicator in the presence of known Ca^{2+} concentrations (to determine Kd).

EXAMPLE DATA ANALYSIS AND FIGURES

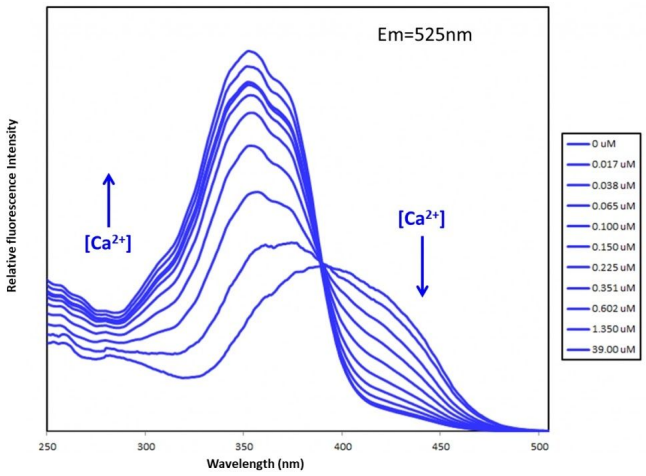

Figure 1. Fluorescence excitation spectra of Fura-10™ in the presence of 0 to 39 µM free Ca2+.

DISCLAIMER

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