Quin-2, AM *CAS 83104-85-2*

Catalog number: 21050
Unit size: 1 mg

Component	Storage	Amount (Cat No. 21050)
Quin-2, AM *CAS 83104-85-2*	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

OVERVIEW

Quin-2 binds calcium tightly and resembles calcium chelator EGTA in ability to bind calcium much more tightly than magnesium. Binding of calcium causes large changes in ultraviolet absorption and fluorescence. The wavelengths of light that cause fluorescence when calcium is bound are longer than the wavelengths that cause fluorescence when it is not bound. When excited at two different wavelengths, the ratio of the fluorescence intensities at the two wavelengths gives the ratio of the concentrations of bound to free calcium. Free Quin-2 concentration can be measured precisely, so free calcium concentration can be calculated precisely. Quin-2 may be injected into cells to measure moment-to-moment changes in intracellular calcium concentration. Quin-2 AM is permeable to cells, and used for studying live cells.

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	475
Emission	495
Excitation	340
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Bottom read mode/Programmable liquid handling

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

Quin-2 AM Stock Solution

1. Prepare a 2 to 5 mM stock solution of Quin-2 AM in high-quality, anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

Quin-2 AM Working Solution

1. On the day of the experiment, either dissolve Quin-2 AM in DMSO or thaw an aliquot of the indicator stock solution to room temperature.

2. Prepare a 2 to 20 µM Quin-2 AM working solution in a buffer of your choice (e.g., Hanks and Hepes buffer) with 0.04% Pluronic® F-127. For most cell lines, Quin-2 AM at a final concentration of 4-5 µM is recommended. The exact concentration of indicators required for cell loading must be determined empirically.

   Note: The nonionic detergent Pluronic® F-127 is sometimes used to increase the aqueous solubility of Quin-2 AM. A variety of Pluronic® F-127 solutions can be purchased from AAT Bioquest.

   Note: If your cells contain organic anion-transporters, probenecid (1-2 mM) may be added to the dye working solution (final in well concentration will be 0.5-1 mM) to reduce leakage of the de-esterified indicators. A variety of ReadiUse™ Probenecid products, including water-soluble, sodium salt, and stabilized solutions, can be purchased from AAT Bioquest.

SAMPLE EXPERIMENTAL PROTOCOL

Following is our recommended protocol for loading AM esters into live cells. This protocol only provides a guideline and should be modified according to your specific needs.

1. Prepare cells in growth medium overnight.

2. On the next day, add 1X Quin-2 AM working solution to your cell plate.

   Note: If your compound(s) interfere with the serum, replace the growth medium with fresh HHBS buffer before dye-loading.

3. Incubate the dye-loaded plate in a cell incubator at 37 °C for 30 to 60 minutes.

   Note: Incubating the dye for longer than 1 hour can improve signal intensities in certain cell lines.

4. Replace the dye working solution with HHBS or buffer of your choice (containing an anion transporter inhibitor, such as 1 mM probenecid, if applicable) to remove any excess probes.
5. Add the stimulant as desired and simultaneously measure fluorescence using a fluorescence plate reader containing a programmable liquid handling system such as a FlexStation, at Ex/Em = 340/495 cutoff 475 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

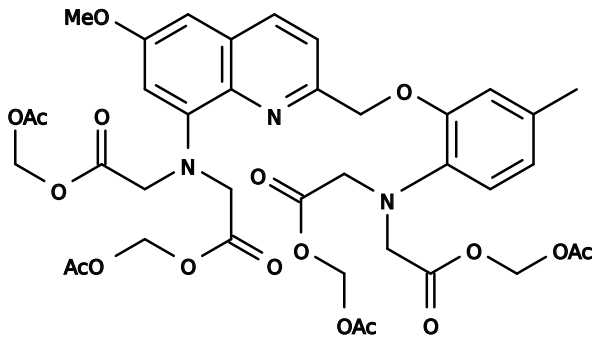

Figure 1. Chemical structure for Quin-2, AM *CAS 83104-85-2*

DISCLAIMER

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