Fura Red, AM *CAS 149732-62-7*

Catalog number: 21046, 21048
Unit size: 1 mg, 10x50 ug

Component	Storage	Amount (Cat No. 21046)	Amount (Cat No. 21048)
Fura Red, AM *CAS 149732-62-7*	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)	10x50 ug

OVERVIEW

Fura Red is a visible light-excitable fura-2 analog that offers unique possibilities for ratiometric measurement of calcium ion in single cells by microphotometry, imaging or flow cytometry when used with single excitation, green-fluorescent calcium indicators. Fura Red AM is the cell-permeable version of Fura Red used for noninvasive intracellular loading. Fura Red AM can be simultaneously loaded into cells with Fluo-3 AM, Fluo-8 AM or Cal-520 AM. An advantage of combining two calcium dyes is that dyes with longer excitation wavelengths can be used. This usually causes less harm to the cells than using ratiometric dyes that are excited with UV- or near UV-light (e.g. Fura-2), as light at visible wavelengths is less phototoxic.

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	Ex/Em = 435/630, cutoff 610. Ex/Em = 470/650, cut off 630
Emission	630, 650
Excitation	435, 470
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

Fura Red AM Stock Solution

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

PREPARATION OF WORKING SOLUTION

Fura Red AM Working Solution

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

2. Prepare a 2 to 20 µM Fura Red 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, Fura Red 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 Fura Red 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 Fura Red 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 monitor fluorescence intensity using a fluorescence plate reader, which contains a programmable liquid handling system such as a FlexStation, at Ex/Em_1 = 435/630 nm cutoff 610 nm and Ex/Em_2 = 470/650 nm cutoff 630 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

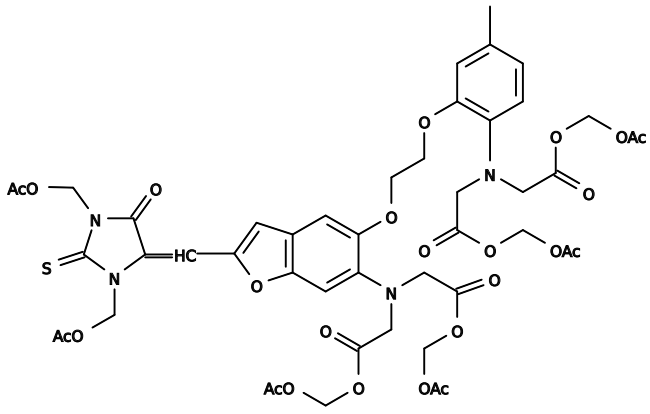

Figure 1. Chemical structure for Fura Red, AM *CAS 149732-62-7*

DISCLAIMER

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