Fura-10™, AM

Catalog number: 21114, 21115
Unit size: 5x50 ug, 1 mg

Component	Storage	Amount (Cat No. 21114)	Amount (Cat No. 21115)
Fura-10™, AM	Freeze (< -15 °C), Minimize light exposure	5x50 ug	1 mg

OVERVIEW

Among ratiometric calcium ion indicators, Fura-2 and Indo-1 are the two most popular ones. However, there are still a few challenges for using these two calcium ion indicators, in particular, for live cells. UV-excitation of Fura 2 caused fast photobleaching. Fura-8™ was introduced a few years ago to shift the excitation closer to visible light. Although Fura-8 demonstrated significant improvement in the ratio of signal/background, it is not well retained in live cells just like Fura-2. Fura-10 have recently been introduced to address this cellular retention issue. Fura 10 demonstrated dramatic improvement in the ratio of signal/background in the absence of probenecid.

KEY PARAMETERS

Fluorescence microplate reader

Excitation	354 nm and 415 nm
Emission	524 nm
Cutoff	475 nm
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-10™ AM stock solution
Prepare a 2 to 5 mM Fura-10™ AM stock solution in high-quality, anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

Fura-10™ AM working solution
On the day of the experiment, either dissolve Fura-10™ AM in DMSO or thaw an aliquot of the indicator stock solution to room temperature. Prepare a dye working solution of 2 to 20 μM in a buffer of your choice (e.g., Hanks and Hepes buffer) with 0.04% Pluronic® F-127. For most cell lines, Fura-10™ 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-10™ 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 solution, 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-10™ AM working solution into 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 containing a programmable liquid handling system such as a FlexStation, at Ex/Em_1 = 354/524 nm cutoff 475 nm and Ex/Em_2 = 415/524 nm cutoff 475 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

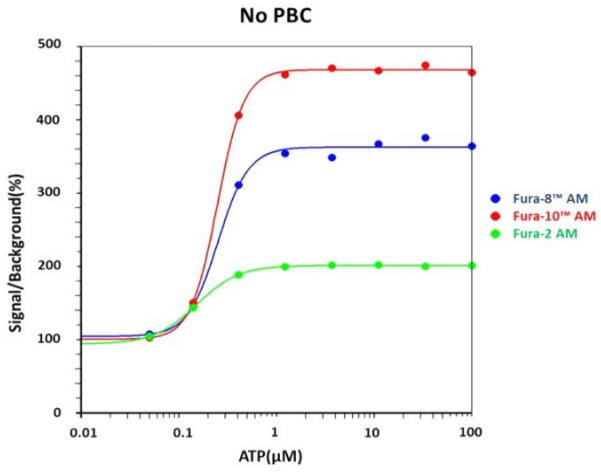

Figure 1. ATP-stimulated calcium response of endogenous P2Y receptor in CHO-K1 cells measured with Fura-2 AM, Fura-8™ AM and Fura-10™ AM in the absence of Probenecid. CHO-K1cells were seeded overnight in 50,000 cells per 100 μL per well in a 96-well black wall/clear bottom costar plate. 100 μL of 5 uM Fura-2 AM or Fura-8™ AM or Fura-10™ AM without probenecid was added into the cells, and the cells were incubated at 37 ° C for 45 minutes and RT for 30 minutes. ATP (50μL/well) was added by FlexStation (Molecular Devices) to achieve the final indicated concentrations.

DISCLAIMER

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