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.

AT A GLANCE

Protocol summary

1. Prepare cells in growth medium
2. Add Fura-10™ AM dye working solution (100 µL/well for 96-well plate or 25 µL/well for 384-well plate)
3. Incubate at 37 °C for 20 minutes to 2 hours followed by incubation at room temperature for 30 minutes
4. Monitor fluorescence intensity at Ex1/Em1 = 354/524 nm and Ex2/Em2 = 415/524 nm

Important
Store at –20 °C, protected from light. Expiration date is 12 months from the date of receipt.

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

Other instruments

FDSS, FLIPR, ViewLux, NOVOStar, ArrayScan, FlexStation, IN Cell Analyzer

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 esters stock solution in high-quality, anhydrous DMSO.

Note Unused Fura-10™ AM stock solution can be aliquoted and stored at -20 °C for more than one month if the tubes are sealed tightly.

Note Protect from light and avoid repeated freeze-thaw cycles.

PREPARATION OF WORKING SOLUTION

Fura-10™ AM working solution
Prepare a working solution of 1 to 20 µM in the buffer of your choice (such as Hanks and Hepes buffer) with 0.04% Pluronic® F-127.

Note For most cell lines we recommend the final concentration of calcium indicators be 4-5 µM. The exact concentration of indicators required for cell loading must be determined empirically.

Note Avoid any artifacts caused by overloading and potential dye toxicity, it is recommended to use the minimal probe concentration that can yield sufficient signal strength.

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

Note If your cells (such as CHO cells) containing the organic anion-transports, no probenecid or lower concentration of (0.2 - 1 mM) probenecid can be used for the cell loading step. A variety of ReadiUse™ probenecid 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.

2. Add equal volume of the Fura-10™ dye working solution into your cell plate.

3. Incubate the dye-loading plate room at temperature or 37 °C for 20 minutes to 2 hours, and then incubate the plate at room temperature for another 30 minutes.

   Note Decreasing the loading temperature might reduce the compartmentalization of the indictor.

   Note Incubation time should be optimized as per the cells type.

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 excess probes.

5. Add the stumilant as desired.

6. Monitor fluorescence intensity at Ex1/Em1 = 354/524 nm and Ex2/Em2 = 415/524 nm (Cutoff = 475 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

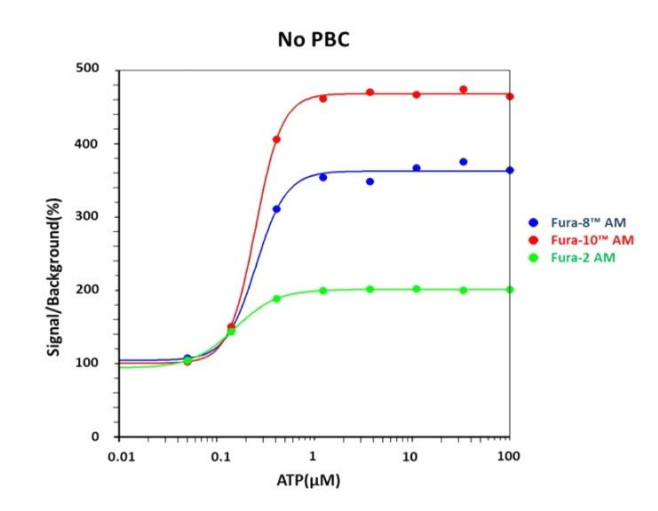

Figure 1. ATP-stimulated calcium response of endogenous P2Y receptor in CHO-K1 cells measured with Fura-2 AM, Fura-8™ AM and Fura-10™ AM in the absence of Probenecid. CHO-K1cells were seeded overnight in 50,000 cells per 100 µL per well in a 96-well black wall/clear bottom costar plate. 100 µL of 5 uM 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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