ION BIOSCIENCES

Advion Interchim scientific

Instructions

ICR-1 AM

Materials Needed

Name	Volume	Containers	Storage
ICR-1 AM (100 µg vial)	Dry	1	-20° C
DMSO	25 µL	1	25° C
100X Pluronic F-127 solution	100 µL	1	4° C
100X Probenecid solution	100 µL	1	4° C
Assay Buffer	10 mL	1	4° C

Description

ION Calcium Red - 1 (ICR-1) is a red fluorescent (Ex/Em 580nm/660nm) calcium (Ca^{2+}) indicator for intracellular Ca^{2+} measurements. ICR-1's long-wavelength emission and a large Stokes shift reduces contributions of autofluorescence, making ICR-1 AM optimal for cellular and tissue imaging applications. ICR-1 can also be multiplexed with GFP-labeled cells or other green fluorophores commonly used in experiments. Unlike some other red fluorescent Ca^{2+} indicators, ICR-1 does not accumulate in the mitochondria. Data supporting fluorescence lifetime imaging and multiphoton imaging capabilities have also been demonstrated.

The recommended protocol recommends enough reagents to make 10 mL of working solution, enough for one 96-well or 384-well plate. The actual number of assays will vary according to optimal dye concentrations for your application.

Laboratory Procedures

General Considerations

Before you begin, make sure that you have all the additional reagents and materials you will need for the successful completion of your experiment. In addition to reagents, a fluorescence microscope or plate reader that can provide an excitation source at ~585 nm and measure emission at ~645 nm is required. The most suitable filter set for imaging applications is Texas Red.

1. Optimal dye concentrations will vary depending on cell type and application. Recommended dye loading concentration is ~10 µM.
2. Aqueous solutions of ICR-1 AM are susceptible to hydrolysis; therefore, all working solutions should be used as quickly as possible and no later than 2 hours after preparation for best results.

Laboratory Procedures (continued)

The following protocol provides general guidelines for using ICR-1 to measure intracellular calcium. Follow suggestions for best results.

1. Allow all reagents to warm to room temperature before proceeding.
2. Add 9.7 mL of Assay Buffer to a conical tube (15 – 50 mL).
3. Add 100 µL of 100X Pluronic F-127 solution (Catalog #: 7601A) to conical tube. Pluronic F-127 is a biocompatible surfactant that aids in dye dissolution, ensuring equitable dye distribution and cellular loading.
4. Add 200 µL of 50X Probenecid solution (Catalog #: 7300P) to conical tube. Probenecid inhibits cellular efflux of many fluorescent ion indicators.[1]
5. Vortex conical tube briefly to mix.
6. Dissolve ICR-1 AM in 25 µL of DMSO. After adding DMSO, vortex tube briefly to dissolve the indicator dye, then centrifuge briefly to collect all contents at the tube bottom. Add entire contents of indicator dye tube to assay buffer solution to make **dye loading solution**.
7. Vortex **dye loading solution** briefly to mix.
8. Remove the cell culture medium from your cells and add **dye loading solution**. Recommended volumes are: 35 mm dish or 6-well plate, 1.5 mL; 96-well plate, 100 µL; 384-well plate, 20 µL.[2]
9. Incubate in a cell culture incubator at 37ºC for ~4 hours.
10. Acquire data using a FlexStation kinetic plate reader (Ex/Em: 585/645 nm, Cutoff: 610 nm) or image using a fluorescence microscope (using Texas Red filters). Begin data acquisition at a 1 Hz frequency, then after 20 seconds add your compounds of interest to the cell-containing plate and continue data acquisition for an additional 90 seconds.

[1]Probenecid is included in the **Dye Loading Solution** to aid dye retention. This may be particularly important in certain cell lines (e.g. CHO cells). However, caution is advised when using Probenecid as it may have undesirable effects on assay performance for the target of interest.

[2]In some cases, such as when using suspension-based cultures, medium aspiration is not desirable. In these circumstances, we recommend doubling the concentration of all reagents in your dye loading buffer and adding an equal volume of dye loading solution to medium to achieve the same final loading concentrations.

Example Results

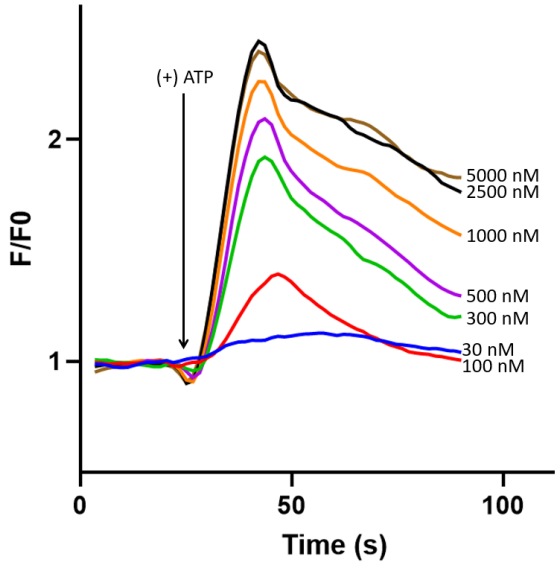

Figure 1. Calcium flux in CHO K1 cells. Kinetic fluorescence data acquired using a Molecular Devices FlexStation® (Ex: 585 nm, Em: 645 nm, Cutoff: 610 nm) for all ATP concentrations evaluated. ATP activates endogenously expressed P2X7 receptors, which leads to increased intracellular calcium, was added at 30 sec. All measurements were acquired at 37°C.

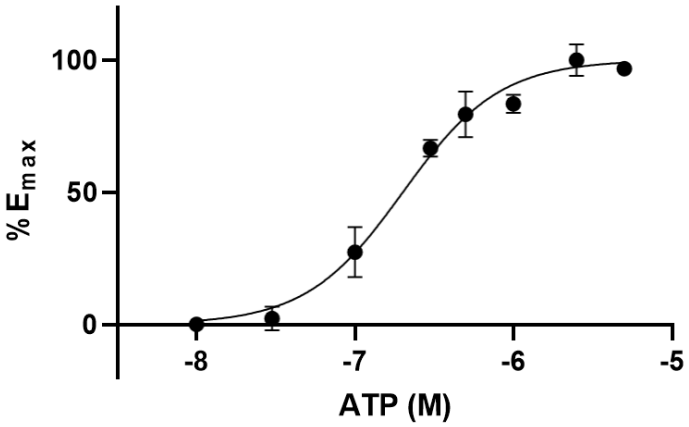

Figure 2. An ATP concentration response curve in CHO K1 cells using ION's Red Calcium Essentials kit. Data was acquired using a Molecular Devices FlexStation® (Ex: 585 nm, Em: 645 nm, Cutoff: 610 nm). All measurements were recorded at 37°C. The estimated EC_{50} is 200 nM, error bars represent standard deviation (n=3)

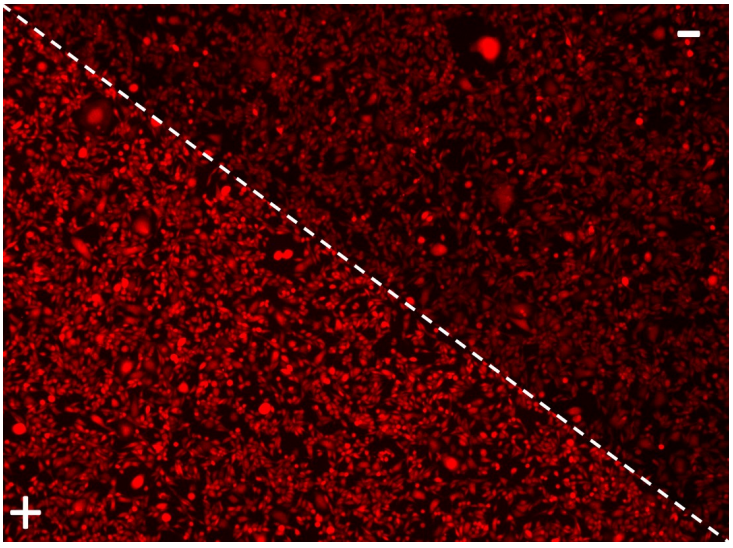

Figure 3. ICR-1 fluorescence in CHO K1 cells in the presence (+) and absence (-) of 500 nM ionomycin. Fluorescence (Ex: 580 nm, Em: 660 nm) was recorded on a BioTek® Cytation 5 plate reader. Images were acquired using Texas Red filters, Ex: 586/15, Em: 647/57 and a 4X objective.

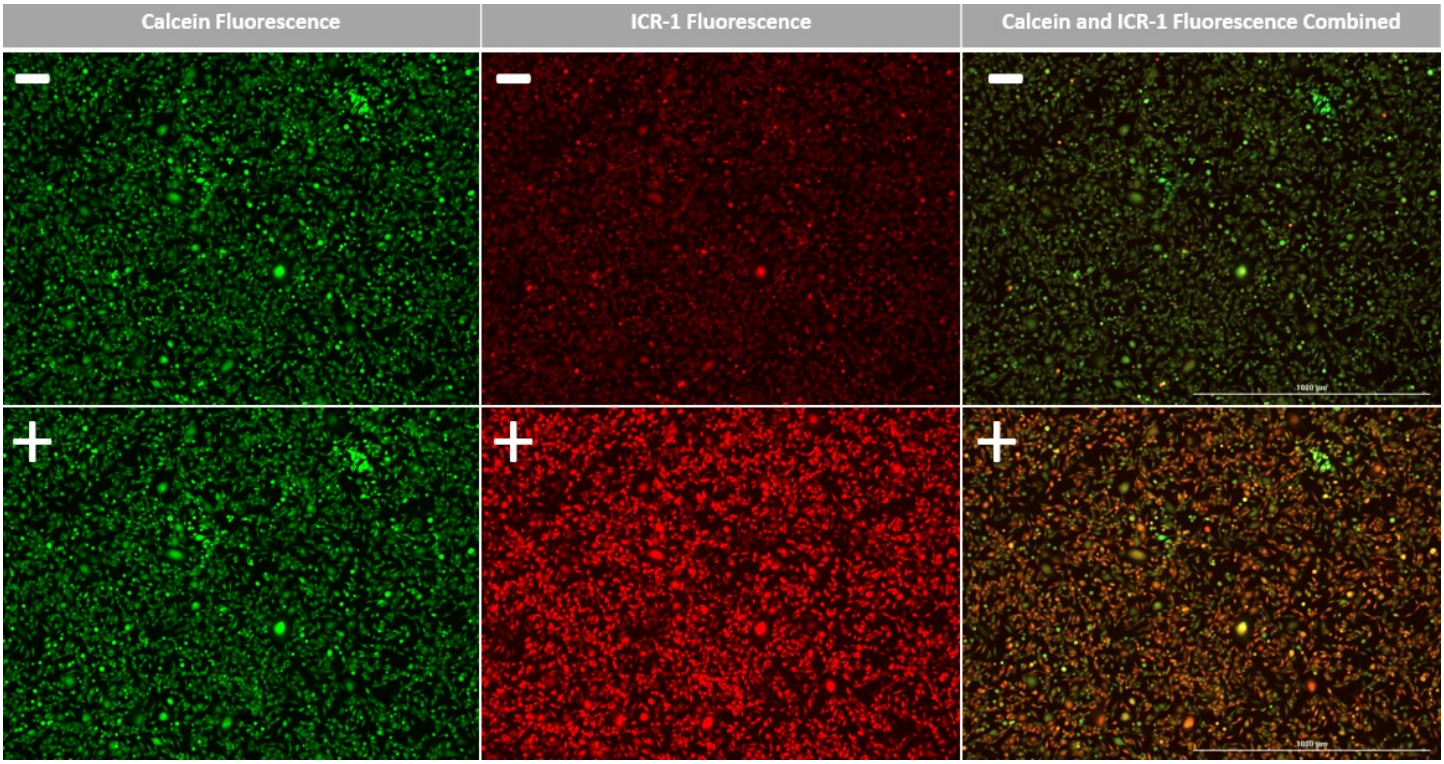

Figure 4. Left column—Calcein, middle column—ICR-1, right column—merged fluorescence in CHO K1 cells in the presence (+) or absence (-) of 500 nM ionomycin. After the addition of ionomycin caused an increase in intracellular calcium, only ICR-1 fluorescence increased. There is no spectral overlap between the indicators. ICR-1 fluorescence (Ex/Em: 580 nm/660 nm) and Calcein fluorescence (Ex/Em: 495 nm/515 nm) were recorded on a BioTek® Cytation 5 plate reader. Images were acquired using Texas Red filters, Ex: 586/15, Em: 647/57 or GFP filters, Ex: 469/35 nm, Em: 525/39 nm, and a 4X objective.

Related Products

Product Code	Product Name
1091C	ICR-1 AM (500 µg)
7601A	Pluronic F-127 (100X)
7300p	Probenecid Solution (50X)