Instructions

MIDSCI™
Laboratory Equipment & Supplies

Ratiometric Calcium *Essentials*

Kit Contents

Name	Volume	Containers	Storage
Fura-2 AM (50 µg vial)	Dry	10	-20° C
50X TRS solution	2 mL	1	4° C
100X Pluronic F-127 solution	1 mL	1	4° C
1X Assay Buffer	100 mL	1	4° C

Description

Ratiometric Calcium Essentials kit provides the necessary reagents for conducting no wash, ratiometric calcium flux assays compatible with plate reader and fluorescence microscopy applications. Individual components are provided to give the user the flexibility needed to customize your assay.

Fura-2 is the most popular UV-excitable, ratiometric green indicator for intracellular calcium (Ca^{2+}) measurements. Ex/Em: 340/505 nm can be used to measure Ca^{2+}-bound Fura-2, and Ex/Em: 380/505 nm can be used to detect Ca^{2+}-free Fura-2. Ratiometry is not only optimal for imaging applications where quantification of intracellular Ca^{2+} concentrations is desired, it also reduces effects of photobleaching, heterogenous dye loading, and variable cell morphology.

When following the recommended protocol, Ratiometric Calcium Essentials provides enough reagents to make 100 mL of working solution, enough for ten 96-well plates. The actual number of assays will vary according to optimal dye concentrations for your application.

Laboratory Procedures

Getting Started

Before you begin, make sure that you have all the additional reagents and materials you will need for the successful completion of your experiment. Although the Ratiometric Calcium Essentials kit contains the key reagents you will need to prepare your cells for analysis, your experiments will likely require other reagents that are not included in your package. Notably, compounds to be tested and solvents (such as DMSO) for the dissolution of these compounds, and reagents necessary for cell culture are not included.

In addition to reagents, a fluorescence microscope or plate reader that can provide an excitation source at ~340 nm and ~380 nm and measure emission at ~505 nm is required.

General Considerations

1. Optimal dye concentrations will vary depending on cell type and application. Recommended dye concentrations range between 1 µM and 10 µM.

General Considerations (continued)

2. Aqueous solutions of Fura-2 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. Alternatively, prepared dye loading solution can be frozen and stored for up to 1 week.

Laboratory Procedures

The following protocol provides general guidelines for using this dye to measure intracellular calcium. All loading conditions (dye concentration, temperature, and time) should be optimized for your specific assay, application, and instrumentation.

1. Allow all reagents to warm to room temperature before proceeding.
2. Add 9.7 mL of 1X 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 TRS solution (Catalog #: 7060S) to conical tube. TRS is a membrane impermeant dye useful for masking extracellular fluorescence.[1]
5. Vortex conical tube briefly to mix.
6. Dissolve Fura-2 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 a 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 60 minutes.
10. Acquire data using a kinetic plate reader (Ex/Em: 340 and 380 nm/505 nm) or image using a fluorescence microscope (using filters for Fura).[3] Begin data acquisition at a 1 Hz frequency, then after 10 seconds add your compounds of interest to the cell-containing plate and continue data acquisition for an additional 90 seconds.

[1]Caution is advised when using TRS 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.

[3]Fura-2 fluorescence is temperature sensitive. Therefore, it is important to maintain a stable temperature during data acquisition. If you want to conduct assays at room temperature, allow your plate to cool on the bench for 20 minutes prior to reading plate.

Example Results

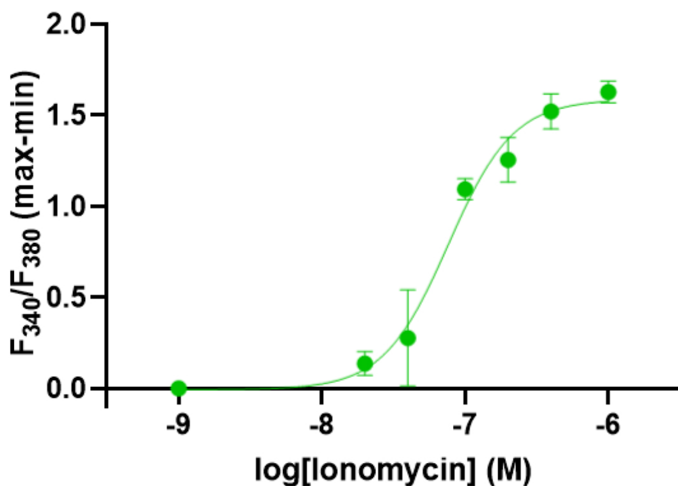

Figure 1. Ionomycin was used to generate a concentration response curve in HeLa cells using Ratiometric Calcium Essentials kit. Data was acquired using a Cytation 5 and Prism software was used to calculate the ratio of F_{340}/F_{380}. All measurements were recorded at 37ºC.

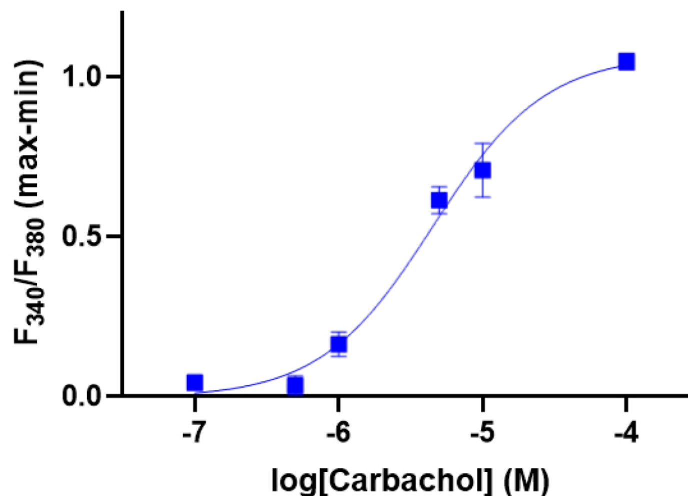

Figure 2. Carbachol was used to stimulate endogenous muscarinic M3 receptor in HEK 293 cells. Data was acquired using a Cytation 5 and Prism software was used to calculate the ratio of F_{340}/F_{380} before and after the addition of carbachol. The EC_{50} is 4.5 µM. All measurements were recorded at 37ºC.

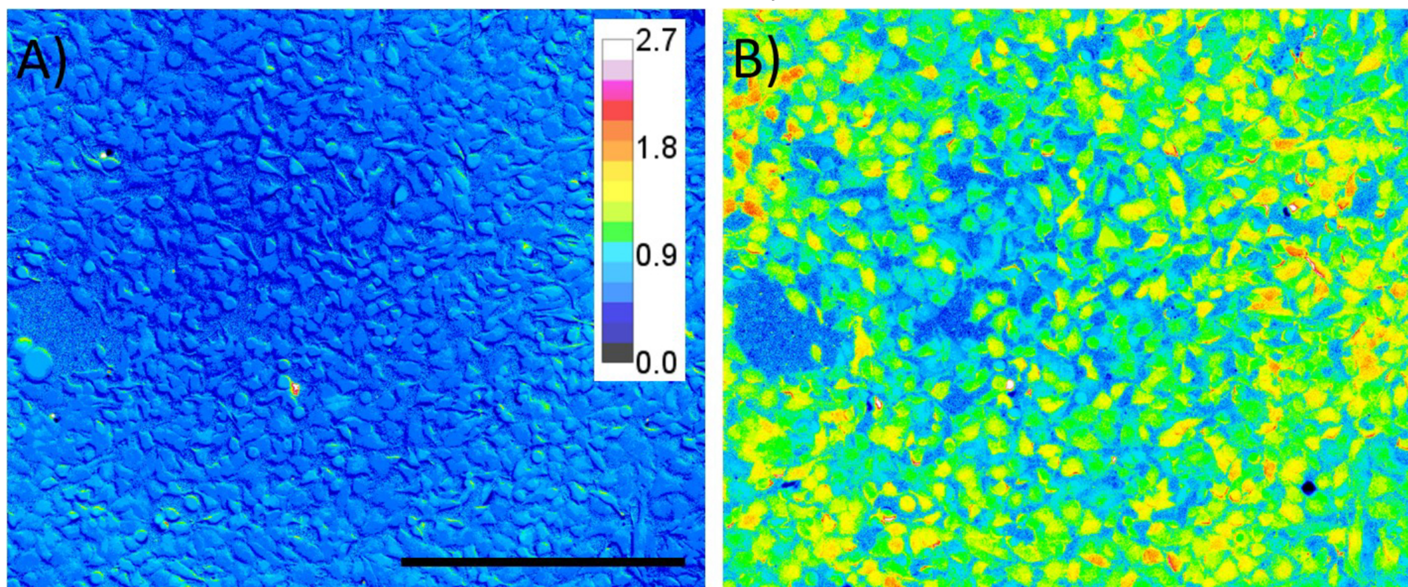

Figure 3. Ratiometric imaging of calcium flux using Ratiometric Calcium Essentials kit. Images of the same region of interest (ROI) were acquired using a 10X objective, excitation filters: (334/40 nm) and (387/11 nm), dichroic mirror (FF409-DiO3), and a single emission filter - (510/80 nm). Corresponding fluorescence images were divided by each other in ImageJ to produce a single image where the colorbar intensity represents the ratio of F_{340}/F_{380}. A) Image of HeLa cells prior to addition of ionomycin. B) Image of the same ROI of HeLa cells after the addition of 500 nM ionomycin. Scale bar - 500 µm.

References

1. Neher E. The use of fura-2 for estimating Ca buffers and Ca fluxes. *Neuropharmacology*. **(1995).** 34(11).
2. Grynkiewicz G, et. al. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem*. **(1985)**. 260(6).
3. Clementi EA, et al. Monitoring changes in membrane polarity, membrane integrity, and intracellular ion concentrations in Streptococcus pneumoniae using fluorescent dyes. *JOVE*. **(2014)**. 84(e51008).

Related Products

Product Code	Product Name
1051	Fura-2 AM (1 mg)
1061	Fura-2 LR AM (1 mg)
7300p	Probenecid Solution (100X)