ION BIOSCIENCES

Instructions

Ratiometric Calcium *Essentials*

Table 1	Kit Contents	*Flex* Kit Cat# 6000-10		*pIONeer* Kit Cat# 6000-2		
Label	Name	Size	Qty	Size	Qty	Storage
Reagent A	Fura-2 AM	50 µg Vial	10	50 µg Vial	2	-20° C
Reagent B	50X TRS[1]	2mL Bottle	1	400 µL Vial	1	4° C
Reagent C	100X Pluronic F-127[2]	1 mL Vial	1	200 µL Vial	1	4° C
Reagent D	1X HEPES-Buffered Hanks Balanced Salt Solution (1X HHBSS)	100 mL Bottle	1	20 mL Bottle	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.
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. Dissolve Fura-2 AM (Reagent A) by adding 25 µL of DMSO to the tube and vortexing until Reagent A is fully dissolved. Centrifuge briefly to collect all contents at the tube bottom.
3. Add the appropriate volume of 1X HHBSS (Reagent D, Table 2) to a 15 mL conical tube.
4. Add 100 µL of 100X Pluronic F-127[2] (Reagent C) to the conical tube in step 3.
5. If desired, add 200 µL of 50X TRS[1] (Reagent B) to the conical tube in step 4.
6. Vortex the conical tube briefly to mix.
7. Add the entire 25 µL of Fura-2 AM Solution from step 2 to the conical tube in step 5.
8. Vortex Dye Loading Solution from step 7 to mix.
6. Remove the cell culture medium from your cells and add the Dye Loading Solution. Recommended volumes are: 1.5 mL for a 35 mm dish or 6-well plate; 100 µL for a 96-well plate; or 20 µL for a 384-well plate.[3]
7. Incubate in a cell culture incubator at 37°C for 60 minutes.
8. 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).[4] 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.

Table 2 Dye Loading Solution

Label	Name	Method A	Method B
Reagent A	Fura-2 AM (DMSO Solution)	25 µL	25 µL
Reagent B	50X TRS[1]	200 µL	-
Reagent C	100X Pluronic F-127[2]	100 µL	100 µL
Reagent D	1X HEPES-Buffered Hanks Balanced Salt Solution (1X HHBSS)	9.7 mL	9.9 mL
	Total	10 mL	10 mL

Table 3	Recommended Instrument Settings
Setting	**Recommendation**
Read Mode (Plate Readers)	'Bottom' read mode only
Ex/Em wavelengths	~340 or 380 nm/505 nm
Cutoff wavelength	495 nm
Filter selection	Fura-2
Contact support@ionbiosciences.com for additional recommendations and guidance on optimizing to your application.	

Laboratory Procedures - Footnotes

[1] Caution is advised when using TRS as it may have undesirable effects on assay performance for the target of interest. TRS contains a membrane-impermeant dye useful for masking extracellular fluorescence.

[2] Pluronic F-127 is a biocompatible surfactant that aids in dye dissolution, ensuring equitable dye distribution and cellular loading.

[3] 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.

[4] 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.

Additional Information

Additional dye indicator and buffer reagents can be purchased either directly from our website or by contacting our Sales Department. Custom and bulk sizes are also available. Contact Sales for more information.

Table 4	Additional Reagents	Available Sizes	
Kit Label	Name	Size	Catalog #
Reagent A	Fura-2 AM	1 mg x 1 Vial	1051B
		50 µg x 20 Vials	1051E
		50 µg x 5 Vials	1051G
Reagent B	50X TRS	20 mL Bottle	7060A
Reagent C	100X Pluronic F-127	10 mL Bottle	7601A
Reagent D	1X HEPES-Buffered Hanks Balanced Salt Solution (1X HHBSS)	20 mL Bottle	7001