

Fura-2 Leakage Resistant (LR) AM

Table 1 - Materials Needed		Most Items Available from ION Biosciences	
Name	Volume	Description/Purpose	
Fura-2 LR AM (50 µg vial)	Dry - 1 Vial	Membrane Permeable Calcium Dye Indicator	
DMSO ¹	25 µL	Solvent for Dissolution of Dye	
100X Pluronic F-127 solution ²	100 µL	Biocompatible Surfactant for Dye Loading	
100X Probenecid solution (optional) ³	100 µL	Intracellular Dye Retention Aid	
Assay Buffer - We Suggest One of the Following:			
⇒ 1X HEPES-Buffered Hank's Balanced Salt Solution	10 mL	1X - Ready to Use Assay Buffer	
⇒ 10X Brilliant Assay Buffer	1 mL	10X - Concentrated Assay Buffer	
Water	As Needed	Dilution of 10X Assay Buffer (if used)	

Description

Fura-2 LR AM is a UV-excitable, ratiometric green indicator for intracellular calcium (Ca^{2+}) measurements with longer cellular retention compared to Fura-2 AM and similar spectral properties. 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 optimal for imaging applications where quantification of intracellular Ca^{2+} concentrations is desired, and reduces effects of photobleaching, heterogeneous dye loading, and variable cell morphology.

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 25 µL DMSO¹ to the tube containing Fura-2 LR AM.
3. Vortex until Fura-2 LR AM is fully dissolved. Centrifuge briefly to collect all contents at the tube bottom.
4. Add the appropriate volume (see **Table 2**, next page) of 1X Assay Buffer to a 15 mL conical tube. If using 10X Assay Buffer, add the appropriate volume of Water (**Table 2**) and 1 mL of 10X Assay Buffer to a 15 mL conical tube.
5. Add 100 µL of 100X Pluronic F-127² solution to the conical tube from **step 4**.

Procedure Continues on Next Page

Laboratory Procedures (continued)

6. (Optional) Add 100 μ L of 100X Probenecid³ solution to the conical tube from **step 5**.
7. Vortex conical tube from **step 6** briefly to mix.
8. Add the entire contents of the Fura-2 LR AM in DMSO solution from **step 3** to the conical tube from **step 7** to make the **Dye Loading Solution⁴**.
9. Vortex the **Dye Loading Solution⁴** from **step 8** briefly to mix.

Table 2	Dye Loading Solution ⁴	1X Assay Buffer		10X Assay Buffer	
Name		Method A	Method B	Method C	Method D
Fura-2 LR AM in DMSO ¹ Solution		25 μ L	25 μ L	25 μ L	25 μ L
100X Pluronic F-127 ² solution		100 μ L	100 μ L	100 μ L	100 μ L
100X Probenecid ³ solution		100 μ L	-	100 μ L	-
1X HEPES-Buffered Hanks Balanced Salt Solution		9.8 mL	9.9 mL	-	-
10X Brilliant Assay Buffer		-	-	1 mL	1 mL
Water		-	-	8.8 mL	8.9 mL
Total		10 mL	10 mL	10 mL	10 mL

10. Remove the cell culture medium and add the **Dye Loading Solution⁴** from **step 9**. Recommend volumes are: 35 mm dish or 6-well plate, 1.5 mL; 96-well plate, 100 μ L; 384-well plate, 20 μ L.⁵
11. Incubate in a cell culture incubator at 37°C for 60 minutes⁶.
12. Conduct a wash⁵ step to remove the **Dye Loading Solution⁴** and replace with cell culture medium or assay buffer. Repeat, if necessary, to completely remove extracellular dye.
13. Acquire data using a fluorescence microscope^{6,7} equipped with Fura-2 filter or a fluorescence plate reader using an excitation wavelength of ~340 or 380 nm, an emission wavelength of ~505 nm and an acquisition frequency of 1-10 Hz⁸. See **Table 3** below for recommended settings.

Table 3	Recommended Instrument Settings
Setting	Recommendation
Read Mode (Plate Readers)	'Bottom' read mode only
Ex/Em wavelengths ⁸	~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 DMSO is hygroscopic and should be stored tightly closed. Wet solvent causes difficulties with dissolution of the dye.
- ² Pluronic F-127 is a biocompatible surfactant that aids in dye dissolution, ensuring equitable dye distribution and cellular loading.
- ³ Probenecid is an anion transport inhibitor that improves intracellular dye retention. Although it is not required for all cell types and dyes, it is recommended in most cases to optimize assay performance.
- ⁴ The Dye Loading Solution should be used within 2 hours of dye addition for best results.
- ⁵ In some cases, a no wash format works best. If a no wash format is indicated for your application, we recommend doubling the concentration of 100X Pluronic F-127 and 100X Probenecid in your dye loading buffer.
- ⁶ Fura-2 fluorescence is temperature sensitive, therefore it is important to maintain a stable temperature during data acquisition. To conduct assays at room temperature, allow the plate to cool on the bench for 20 minutes prior to reading the plate.
- ⁷ (Optional) To minimize extracellular background, dye loading solution can be replaced with assay buffer containing 1X TRS solution. TRS is a membrane impermeant dye useful for masking extracellular fluorescence. Caution is advised when using TRS or any other extracellular masking solutions as they may have undesirable effects on assay performance for the target of interest.
- ⁸ To prevent bleed-through or spectral overlap, the Ex/Em wavelengths may need to be optimized by broadening the interval between the wavelengths.

Additional Information

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	Available Reagents	Available Sizes	
Name		Size	Catalog #
Fura-2 Leakage Resistant (LR) AM		1 mg x 1 Vial	1061B
		50 µg x 20 Vials	1061E
		50 µg x 5 Vials	1061G
100X Pluronic F-127 Solution		10 mL Bottle	7601A
100X Probenecid Solution		10 mL Bottle	7300P-100
1X HEPES-Buffered Hank's Balanced Salt Solution (1X HHBSS)		100 mL Bottle	7001
10X Brilliant Assay Buffer		10 mL Bottle	7010X
50X TRS Solution		20 mL Bottle	7060A