ICR-1 AM

Table 1 - Materials Needed	Most Items Available from ION Biosciences	
Name	**Volume**	**Description/Purpose**
ICR-1 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]	50 µL	Biocompatible Surfactant for Dye Loading
100X Probenecid solution (optional)[3]	50 µL	Intracellular Dye Retention Aid
Assay Buffer - We Suggest One of the Following:		
⇒ 1X HEPES-Buffered Hank's Balanced Salt Solution	5 mL	1X - Ready to Use Assay Buffer
⇒ 10X Brilliant Assay Buffer	0.5 mL	10X - Concentrated Assay Buffer
Water	As Needed	Dilution of 10X Assay Buffer (if used)

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.

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. Recommended dye loading concentration for ICR-1 AM is ~10 µM.

1. Allow all reagents to warm to room temperature before proceeding.
2. Add 25 µL DMSO[1] to the tube containing ICR-1 AM.
3. Vortex until ICR-1 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 50 µL of 100X Pluronic F-127[2] solution to the conical tube from **step 4**.

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Laboratory Procedures (continued)

6. (Optional) Add 50 µL of 100X Probenecid[3] 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 ICR-1 AM in DMSO solution from **step 3** to the conical tube from **step 7** to make the **Dye Loading Solution**[4].
9. Vortex the **Dye Loading Solution**[4] 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**
ICR-1 AM in DMSO[1] solution	25 µL	25 µL	25 µL	25 µL
100X Pluronic F-127[2] solution	50 µL	50 µL	50 µL	50 µL
100X Probenecid[3] solution	50 µL	-	50 µL	-
1X HEPES-Buffered Hanks Balanced Salt Solution	4.9 mL	4.95 mL	-	-
10X Brilliant Assay Buffer	-	-	0.5 mL	0.5 mL
Water	-	-	4.4 mL	4.45 mL
Total	5 mL	5 mL	5 mL	5 mL

10. Remove the cell culture medium and add the **Dye Loading Solution**[4] from **step 9**. Recommend volumes are: 35 mm dish or 6-well plate, 1.5 mL; 96-well plate, 100 µL; 384-well plate, 20 µL.[5]
11. Incubate in a cell culture incubator at 37°C for ~ 4 hours.
12. Conduct a wash[5] step to remove the **Dye Loading Solution**[4] 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] equipped with Texas Red filter or a fluorescence plate reader using an excitation wavelength of ~585 nm, an emission wavelength of ~645 nm and an acquisition frequency of 1-10 Hz[6]. 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[6]	~585 nm/645 nm
Cutoff wavelength	610 nm
Filter selection	Texas Red
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] 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 #
ICR-1 AM		500 µg x 1 Vial	1091C
		50 µg x 10 Vials	1091F
		50 µg x 3 Vials	1091G
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