

FLUO-4 AM Ultrapure (>98%)

Table 1 - Materials Needed		Most Items Available from ION Biosciences
Name	Volume	Description/Purpose
Fluo-4 AM Ultrapure (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
50X TRS (optional) ⁴	200 µL	Extracellular Fluorescence Masking Agent
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

Fluo-4 Ultrapure is the same green fluorescent, intracellular calcium (Ca²⁺) indicator that's been proven and trusted for decades, but with minimal impurities to give you maximum confidence. The most popular solution to measure intracellular Ca²⁺ flux in high throughput screening and fluorescence microscopy applications.

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 Fluo-4 AM Ultrapure.
3. Vortex until Fluo-4 AM Ultrapure 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 3**, next page) 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**.

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Laboratory Procedures (continued)

6. (Optional) Add 100 μ L of 100X Probenecid³ solution to the conical tube from **step 5**.
7. (Optional) Add 200 μ L of 50X TRS⁴ solution to the conical tube from **step 6**.
8. Vortex conical tube from **step 7** briefly to mix.
9. Add the entire contents of the Fluo-4 AM Ultrapure in DMSO solution from **step 3** to the conical tube from **step 8** to make the **Dye Loading Solution⁵**.
10. Vortex the **Dye Loading Solution⁵** from **step 9** briefly to mix.

Table 2	Dye Loading Solution ⁵	Using 1X Assay Buffer			
		Method A	Method B	Method C	Method D
	Fluo-4 AM Ultrapure 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 (optional)	100 μ L	100 μ L	-	-
	50X TRS ⁴ (optional)	200 μ L	-	200 μ L	-
	1X HEPES-Buffered Hanks Balanced Salt Solution	9.6 mL	9.8 mL	9.7 mL	9.9 mL
	Total	10 mL	10 mL	10 mL	10 mL

Table 3	Dye Loading Solution ⁵	Using 10X Assay Buffer			
		Method A	Method B	Method C	Method D
	Fluo-4 AM Ultrapure 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 (optional)	100 μ L	100 μ L	-	-
	50X TRS ⁴ (optional)	200 μ L	-	200 μ L	-
	10X Brilliant Assay Buffer	1 mL	1 mL	1 mL	1 mL
	Water	8.6 mL	8.8 mL	8.7 mL	8.9 mL
	Total	10 mL	10 mL	10 mL	10 mL

Procedure Continues on Next Page

Laboratory Procedures (continued)

11. Remove the cell culture medium and add the **Dye Loading Solution**⁵ from **step 10**. Recommend volumes are: 35 mm dish or 6-well plate, 1.5 mL; 96-well plate, 100 µL; 384-well plate, 20 µL.⁶
12. Incubate in a cell culture incubator at 37°C for 60 minutes.
13. Conduct a wash⁶ step to remove the **Dye Loading Solution**⁵ and replace with cell culture medium or assay buffer. Repeat, if necessary, to completely remove extracellular dye.
14. Acquire data using a fluorescence microscope⁷ equipped with GFP or FITC filters or a fluorescence plate reader using an excitation wavelength of ~490 nm, an emission wavelength of ~520 nm and an acquisition frequency of 1-10 Hz⁸. See **Table 4** below for recommended settings.

Table 4 Recommended Instrument Settings

Setting	Recommendation
Read Mode (Plate Readers)	'Bottom' read mode only
Ex/Em wavelengths ⁸	~490 nm/520 nm
Cutoff wavelength	515 nm
Filter selection	GFP, FITC

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 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.
- ⁵ The Dye Loading Solution should be used within 2 hours of dye addition for best results.
- ⁶ 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, 100X Probenecid, and 50X TRS in your dye loading buffer.
- ⁷ To minimize extracellular background, the dye loading solution can be replaced with assay buffer containing 1X Probenecid solution and/or 1X TRS solution.
- 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
	Fluo-4 AM Ultrapure (>98%)	500 µg x 1 Vial	1043C
		50 µg x 10 Vials	1043F
		50 µg x 3 Vials	1043G
	100X Pluronic F-127 Solution	10 mL Bottle	7601A
	100X Probenecid Solution	10 mL Bottle	7300P-100
	50X TRS Solution	20 mL Bottle	7060A
	1X HEPES-Buffered Hank's Balanced Salt Solution (1X HHBSS)	100 mL Bottle	7001
	10X Brilliant Assay Buffer	10 mL Bottle	7010X