

## Fluo-2 AM

*Fluorescent indicator to assay intracellular free calcium. Membrane-permeable. Higher calcium affinity than Fluo-4, with superior intracellular loading.*

### Product Information

<b>Name :</b>	<b>Fluo-2 AM</b>
<b>Catalog Number :</b>	FP-APINSA, 1 mg APINSB, 20 x 50 µg
<b>CAS:</b>	108964-32-5
<b>Structure :</b>	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>
<b>Molecular Weight :</b>	MW= 1061
<b>Soluble:</b>	DMSO
<b>Absorption / Emission :</b>	$\lambda_{exc}/\lambda_{em} = 495 / 515$ nm
<b>Kd:</b>	290 nM

**Storage:** Store at -20°C (K). Protect from light and moisture  
 Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20°C after preparation. Avoid repeated freeze-thaw cycles.

### Introduction

Fluo-2 is a green fluorescent, intracellular calcium (Ca<sup>2+</sup>) indicator. Fluo-2 has higher Ca<sup>2+</sup> affinity (K<sub>d</sub> = 290nM), superior cell loading and comparable spectral properties compared to Fluo-4. Offers superior sensitivity versus other Fluo analogues at lower Ca<sup>2+</sup> concentrations.

### Directions for use

#### Materials Needed

<u>Name</u>	<u>Volume</u>	<u>Containers</u>	<u>Storage</u>
Fluo-2 AM (50 µg vial)	Dry	1	-20°C
DMSO	25 µL	1	20-25°C
100X Pluronic F-127 solution	100 µL	1	4°C
100X Probenecid solution (optional)	100 µL	1	4°C
100X TRS solution (optional)	100 µL	1	4°C

#### 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 10 mL of assay buffer to a conical tube (15 – 50 mL). HEPES-buffered Hank's Balanced Salt Solution (pH = 7.2-7.4) is the most used assay buffer, although other buffers can also be used.
3. Add 100 µL of 100X Pluronic F-127 solution to conical tube. Pluronic F-127 is a biocompatible surfactant that aids in dye dissolution, ensuring equitable dye distribution and cellular loading.
4. (Optional) Add 100 µL of 100X Probenecid solution to conical tube. 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.<sup>1</sup>

#### FT-APINSB

5. (Optional) Add 100  $\mu$ L of TRS solution (Catalog #: BVUSM0). TRS is a membrane impermeant dye useful for masking extracellular fluorescence.<sup>1</sup>
6. Vortex conical tube briefly to mix.
7. Dissolve Fluo-2 AM in 25  $\mu$ L 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.<sup>2</sup>
8. Vortex dye loading solution briefly to mix.
9. Remove the cell culture medium and add dye loading solution. Recommend volumes are: 35 mm dish or 6-well plate, 1.5 mL; 96-well plate, 100  $\mu$ L; 384-well plate, 20  $\mu$ L.<sup>3</sup>
10. Incubate in a cell culture incubator at 37°C for 60 minutes.
11. Read fluorescence using a plate reader (Ex/Em: 490 nm/515 nm) or image using a fluorescence microscope (using filters for fluorescein or GFP).<sup>4</sup>

<sup>1</sup> Caution is advised when using Probenecid and/or TRS as they may have undesirable effects on assay performance for the target of interest.

<sup>2</sup> The dye loading solution should be used within 2 hours of dye addition for best results.

<sup>3</sup> 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 all reagents in your dye loading buffer.

<sup>4</sup> To minimize extracellular background, dye loading solution can be replaced with assay buffer containing 1X probenecid solution (optional) and/or 1X TRS solution (optional).

## References

- **Amaral M. et al.**, Synthetic Analogues of Gibbilibol B Induce Bioenergetic Damage and Calcium Imbalance in *Trypanosoma cruzi*, *Life*, 13(3), 663 (2023)

## Related Products

- Pluronic® F-127, FP-69806A
- TRS solution, BVUSM0
- Probenecid, Cell Culture Tested, FP-288652
- DMSO, anhydrous, FP-JW7390

## Ordering information

Catalog size quantities and prices may be found at <http://www.fluoprobes>.

Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes / Interchim; Hotline : +33(0)4 70 03 73 06

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