

ING-2 AM

Materials Needed

Name	Volume	Containers	Storage
ING-2 AM (50 µg vial)	Dry	1	-20° C
DMSO	25 µL	1	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

Description

ION Natrium Green - 2 (ING-2) is a yellow-green fluorescent, intracellular sodium (Na^+) indicator with improved cellular loading and significantly higher brightness than SBFI. Its spectral properties (Ex/Em: 525 nm/545 nm) and large dynamic range make ING-2 the best Na^+ indicator for high-throughput screening applications targeting Na^+ channels, and non-selective monovalent cation channels. ING-2 has a more physiologically relevant affinity ($K_d = 20 \text{ mM}$) than ING-1 ($K_d = 92 \text{ mM}$). Also compatible with fluorescence microscopy using common fluorescein, GFP or more ideally YFP filters.

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 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 (Catalog #: 7601A) 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 (Catalog #: 7300A) 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.¹
5. (Optional) Add 100 µL of TRS solution (Catalog #: 7060A). TRS is a membrane impermeant dye useful for masking extracellular fluorescence.¹

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

Laboratory Procedures (continued)

6. Vortex conical tube briefly to mix.
7. Dissolve ING-2 AM in 25 μ 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.²
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 μ L; 384-well plate, 20 μ L.³
10. Incubate in a cell culture incubator at 37°C for 60 minutes.
11. Read fluorescence using a plate reader (Ex/Em: 525 nm/545 nm) or image using a fluorescence microscope (using filters for YFP, GFP or fluorescein).⁴

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

³In 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.

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