

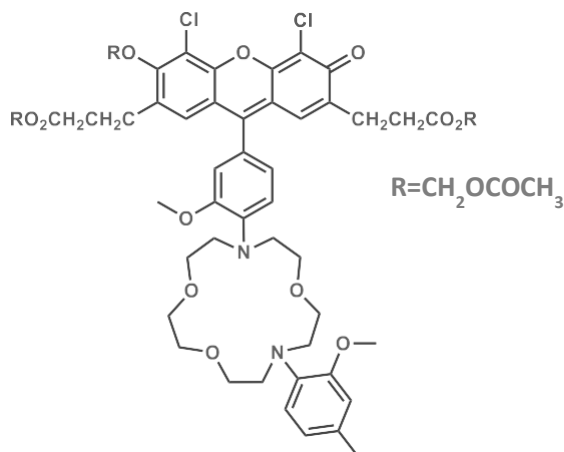
ING-2 AM

05/21

ALTERNATE NAMES: Sodium (Na^+) indicator; ING-2 membrane permeable; ING-2 acetoxymethyl ester; Fluorescent Sodium Na^+ indicator

CATALOG #: B3137-5PK 5 x 50 μg
B3137-500 500 μg

STRUCTURE:



MOLECULAR FORMULA: $\text{C}_{53}\text{H}_{60}\text{Cl}_2\text{N}_2\text{O}_{18}$

MOLECULAR WEIGHT: 1084

APPEARANCE: Orange powder

PURITY: $\geq 90\%$

SOLUBILITY: ~2 mg/ml in DMSO

DESCRIPTION: ING-2 AM is the membrane permeable form of the sodium indicator ING-2 (Cat. No. B3138). Masking the negative charge using non-polar, ester-linked moieties (AM ester) allows the molecule to enter cells through passive diffusion. Once inside the cell, ubiquitous intracellular esterase enzymes promote rapid hydrolysis of the AM ester leaving the active, highly polar form of the indicator trapped inside the cell. ING-2 AM is compatible with spectrofluorimeters and fluorescence microscopy using common fluorescein, GFP or YFP filters.

STORAGE TEMPERATURE: -20 °C. Store in the dark. Product is light sensitive. Protect from air. Store under desiccating conditions.

HANDLING: Do not take internally. Wear gloves and mask when handling the product! Avoid contact by all modes of exposure.

PROTOCOL:

User Supplied Reagents:

- DMSO
- Pluronic F-127 solution
- Probenecid solution (optional)
- TRS solution (optional)
- HEPES-buffered Hank's Balanced Salt Solution

Procedure:

The following protocol provides general guidelines. All the loading conditions (concentrations, temperature and time) should be determined for specific assays, applications, and instrumentation.

1. Allow all reagents to warm to room temperature before proceeding.
2. Add 10 ml of assay buffer to a 15 ml conical tube. HEPES-buffered Hank's Balanced Salt Solution (pH 7.2-7.4) is the most commonly used assay buffer, although other buffers can also be used.

3. Add Pluronic F-127 solution to the assay buffer in conical tube. Pluronic F-127 is a biocompatible surfactant that aids in dye dissolution, ensuring equitable dye distribution and cellular loading.
4. Optional: Add Probenecid solution to the assay buffer. 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.
5. Optional: Add TRS solution. TRS is a membrane impermeant dye useful for masking extracellular fluorescence.
 Note: Caution is advised when using probenecid and/or TRS as they may have undesirable effects on assay performance for the target of interest.
6. Vortex the conical tube briefly to mix.
 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. The dye loading solution should be used within 2 h of dye addition for best results.
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. When cells are sensitive to solution exchanges and will either be aspirated (suspension cells) or detach (loosely adherent cells) during wash steps, we recommend performing assays without removing any solution (no wash format) by adjusting the concentrations of reagents in the dye loading solution so that when everything is added to the wells, the final concentrations of all reagents are the same. If a no wash format is indicated for your application, we recommend doubling the concentration of all reagents in your dye loading buffer.
10. Incubate in a cell culture incubator at 37 °C for 60 min.
11. Read the fluorescence using a plate reader (Excitation/Emission: 525 nm/545 nm) or image using a fluorescence microscope (using filters for YFP, GFP or fluorescein). 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

1. Iamshanova, O., Mariot, P., Lehen'kyi, V., et al. Comparison of fluorescence probes for intracellular sodium imaging in prostate cancer cell lines. *Eur Biophys J* 45, 765–777 (2016).
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5. Aliotta, A., Calderara, D.B., Alberio, L. Flow Cytometric Monitoring of Dynamic Cytosolic Calcium, Sodium, and Potassium Fluxes Following Platelet Activation. *Cytometry*, 97: 933-944 (2020).
6. Naumann, G., Lippmann, K, Eilers, J., et al. Photophysical properties of Na⁺-indicator dyes suitable for quantitative two-photon fluorescence-lifetime measurements. *Journal of Microscopy* 272 (2) 136-144 (2018).

RELATED PRODUCTS:

FURA-2 Am (Cat. No. 2243)
 FURA-5F/AM (Cat. No. 9551)
 FURA-4F/AM (Cat. No. 9550)
 BAPTA AM (Cat. No. 2242)
 ING-2 TMA⁺ Salt (Cat. No. B3138)

DISCLAIMER:

FOR RESEARCH USE ONLY! Not to be used on humans.