ING-1 AM

Materials Needed

Name	Volume	Containers	Storage
ING-1 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 - 1 (ING-1) is a yellow-green fluorescent, intracellular sodium (Na^+) indicator. ING-1 (K_d ~ 92 mM) is a lower affinity sodium indicator than ING-2, with identical spectral properties (Max Ex/Em: 514 nm/542 nm). ING-1 is suitable for measuring changes in intracellular sodium concentrations, and is compatible with fluorescence microscopy, HTS, and fluorescence plate readers using common fluorescein, GFP or more ideally YFP filters.

Laboratory Procedures

The following protocol provides general guidelines for using this dye to measure intracellular sodium. 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]

[1]Caution 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-1 AM in 25 µL 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 µL; 384-well plate, 20 µL.[3]
10. Incubate in a cell culture incubator at 37ºC for 60 minutes.
11. Prepare compound solution(s) and vehicle controls at appropriate concentrations in HHBSS, or similar buffer. We recommend a 3X concentration of compound solution(s) when using volumes suggested in this protocol.
12. Transfer the dye-loaded, cell-containing microplate from **step 10** to your instrument of choice. Acquire baseline fluorescence data (F_0) for each well prior to adding compound solution(s) using an excitation wavelength of ~520 nm, and emission wavelength of ~550 nm.
13. Add 10 µL (for a 384-well plate) of compound solution(s) prepared in **step 11** to the cell-containing plate. Wait 5 - 60 minutes before proceeding to the next step. Wait time will need to be optimized for each assay.
14. Acquire fluorescence data (F) for each well using an excitation wavelength of ~520 nm, and emission wavelength of ~550 nm. Do not change acquisition settings from those used in **step 12** if baseline fluorescence (F_0) data was acquired.

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

[3]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.

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

Example Results

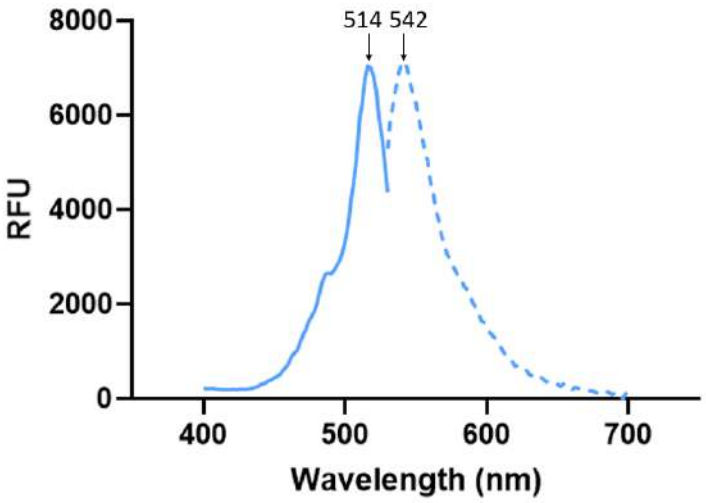

Figure 1. Spectral data of ING-1. Data was acquired with a BioTek® Cytation 5 plate reader. Maximum excitation is at 514 nm and maximum emission is at 542 nm.

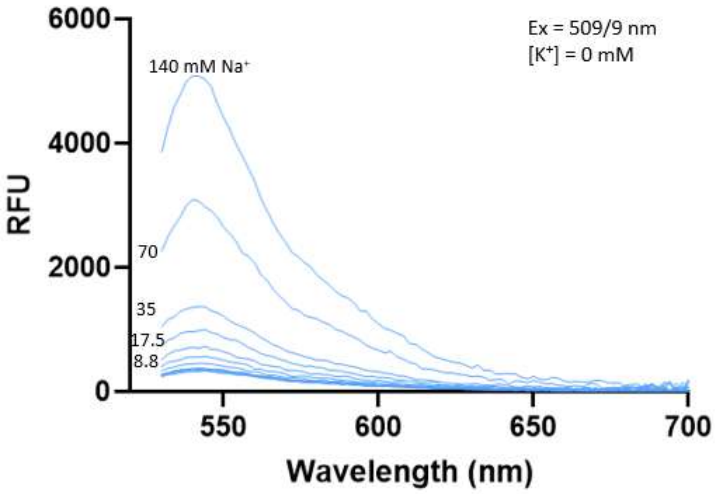

Figure 2. Emission spectral response of ING-1 to Na^+ in K^+-free TRIS buffer (10 mM, pH = 7.4). Data was acquired with a BioTek® Cytation 5 plate reader. Ionic strength was maintained using TMACl.

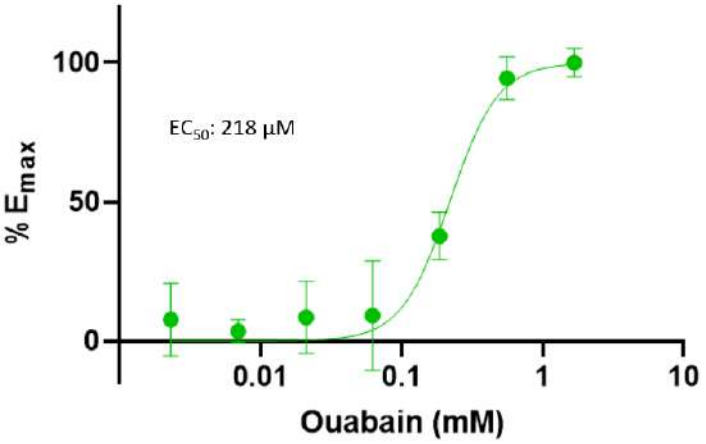

Figure 3. Measuring Na^+/K^+-ATPase inhibition using ING-1. Ouabain concentration response curve (CRC) in CHO K1 (WT) cells measured using ING-1. Fluorescence (Ex: 525/9 nm, Em: 545/9 nm) was recorded using a BioTek® Cytation 5 plate reader 60 min. after the addition of ouabain, and (F_{max}/F_0) values were obtained. The estimated EC_{50} is 218 µM. Error bars represent standard deviation (n = 3).