ION BIOSCIENCES

Instructions

IPG-2 AM

Materials Needed

Name	Volume	Containers	Storage
IPG-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 Potassium Green - 2 (IPG-2) is a yellow-green fluorescent, intracellular potassium (K^+) indicator with Ex/Em: 525 nm/545 nm and a high-sensitivity to detect small changes in K^+ concentration. IPG-2 has a higher affinity (Kd = 18 mM) than IPG-1 (Kd = 50 mM) and lower affinity than IPG-4 (Kd = 7 mM).

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]

[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 IPG-2 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. 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]

[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).