ION BIOSCIENCES

Advion Interchim scientific

Instructions

IPG-4 AM

snapshot compatible

Materials Needed

Name	Volume	Containers	Storage
IPG-4 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 TRS solution (optional)	100 µL	1	4° C
1X Assay Buffer	10 mL	1	4° C

Description

ION Potassium Green - 4 (IPG-4) is a yellow-green fluorescent, intracellular potassium (K^+) indicator with Ex/Em: 525 nm/545 nm with high selectivity for K^+ over Na^+ (8:1). IPG-4 is best suited to detect large changes in intracellular K^+ concentration. IPG-4 is our highest affinity potassium indicator (K_d ~ 7 mM).

IPG-4 is not an MDR1 (P-glycoprotein) substrate; therefore, it can be used to conduct probenecid-free assays.

IPG-4 salt, the membrane-impermeable variant, is best suited to detect small changes in K^+ concentrations in low concentration K^+ environments, like extracellular medium. The salt variant is commonly used to monitor changes in extracellular K^+ in microbial culture systems.

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). We recommend using ION's Brilliant Thallium Assay buffer for studies with IPG-4 AM. HEPES-buffered Hank's Balanced Salt Solution (pH = 7.2 – 7.4) and 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. (Recommended) Add 100 µL of TRS solution (Catalog #: 7060A). TRS is a membrane impermeant dye useful for masking extracellular fluorescence.[1]
5. Vortex conical tube briefly to mix.

[1]Caution is advised when using TRS as it may have undesirable effects on assay performance for the target of interest.

Laboratory Procedures (continued)

6. Dissolve IPG-4 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]
7. Vortex dye loading solution briefly to mix.
8. 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]
9. Incubate in a cell culture incubator at 37°C for 60 - 90 minutes.
10. 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]
11. Add compounds of interest to cell-containing well plate at desired concentrations.
12. Reacquire fluorescence data using the same instrument used in step 10. This step can be performed up to 1 hour after compound addition. *Note: The timing of this acquisition step will need to be optimized for your cell type and target, and will depend on the K^+ efflux kinetics of your assay. It is recommended to acquire kinetic data at 5 min intervals for up to 1 hour whenever possible to optimize read timing for each assay.

[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 TRS solution (optional).

Example Results

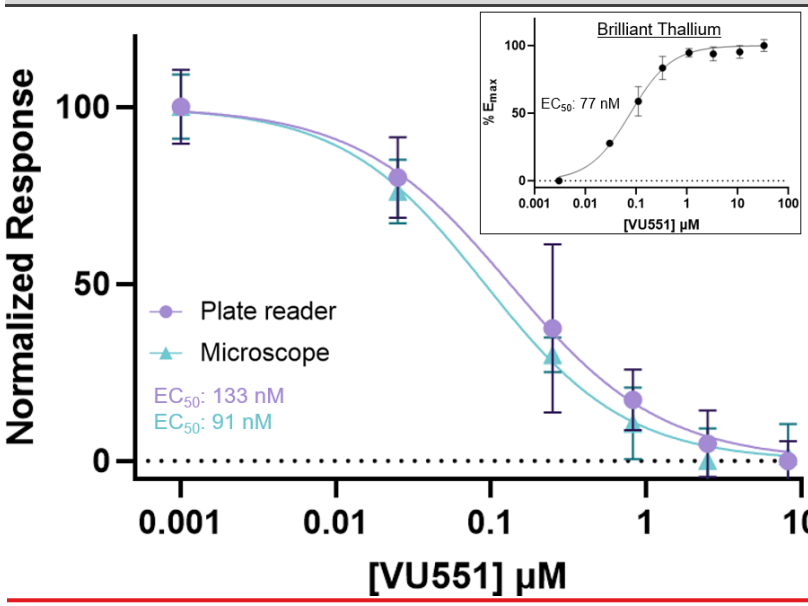

Figure 1. VU551 dose response in GIRK-expressing CHO cells measured using IPG-4. Using ION's patent-pending Snapshot assay protocol, comparable results are obtained whether you use a fluorescence plate reader or microscope. Reported EC_{50} values are in line with values obtained using ION's Brilliant Thallium Assay (inset) on a Flexstation and literature reported values. See Figures 2 and 3 for additional supporting data.

Example Results (continued)

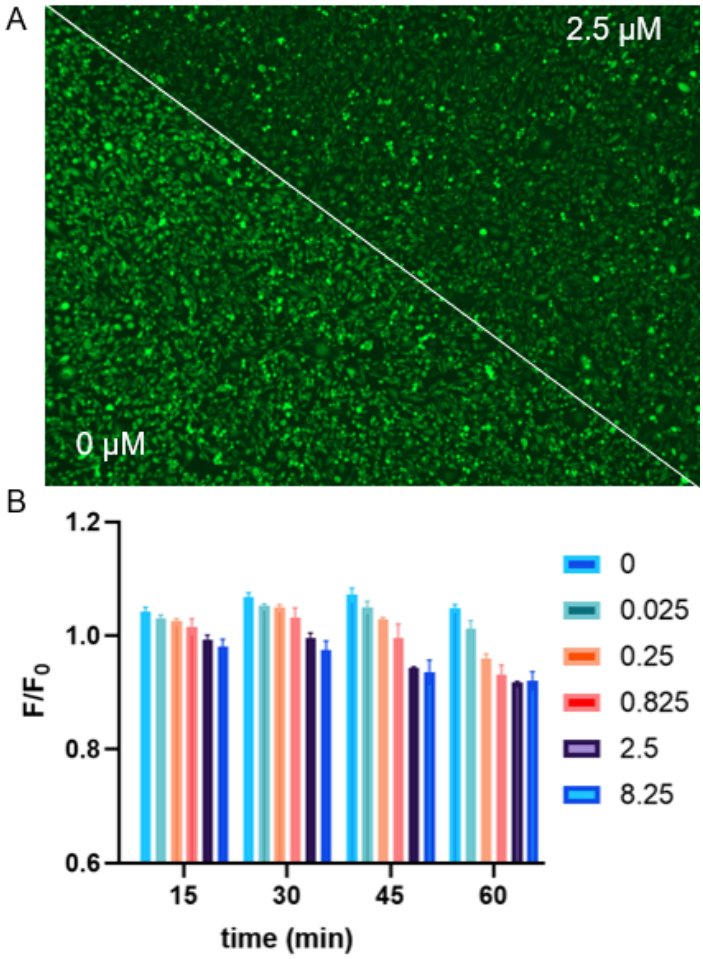

Figure 2. Snapshot assay using IPG-4 with a fluorescence microscope. A) Representative fluorescence images of CHO cells overexpressing GIRK channels acquired 60 min after the addition of vehicle (left) or 2.5 µM VU551, a potent activator of GIRK1/2 (right). GIRK activation results in a reduced concentration of intracellular K^+, leading to a lower fluorescence of IPG-4. Images were acquired using a Cytation 5 plate reader, 4X objective, and GFP filter cube. B) Change in mean cell fluorescence values (F/F_0) of cells exposed to various concentrations of VU551 (µM). The same fields of view were imaged at each time point, and automated analysis was used to quantify mean cell fluorescence intensity in each frame. Error bars represent standard deviation (n = 3 frames).

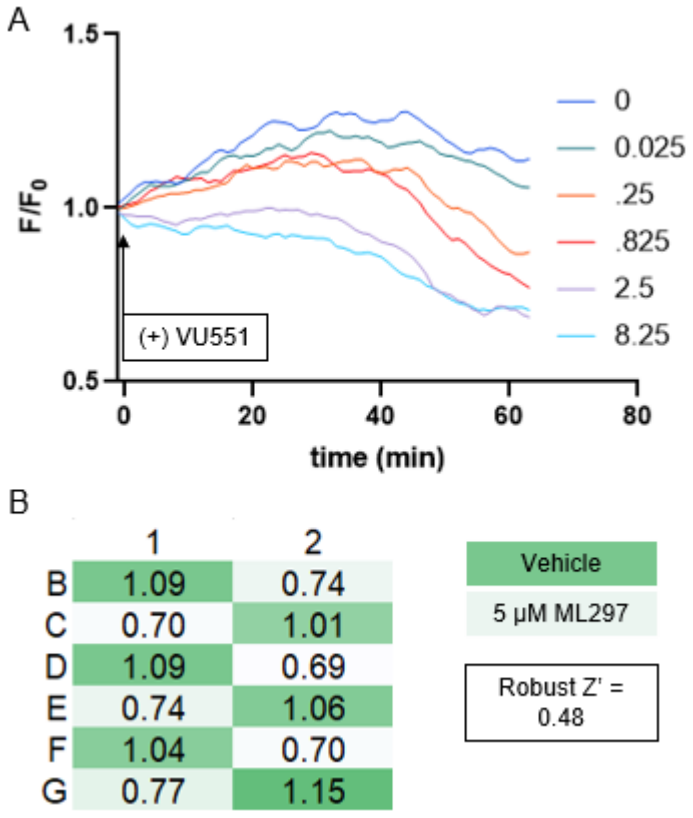

Figure 3. Snapshot assay using IPG-4 with a standard plate reader. A) Change in fluorescence of IPG-4 loaded, CHO cells overexpressing GIRK channels after the addition of VU551, a potent activator of GIRK1/2. GIRK activation results in a reduced concentration of intracellular K^+, leading to a lower fluorescence of IPG-4. Data was acquired using a Cytation 5 plate reader (Ex: 515(9) nm, Em: 545(15) nm). B) Calculated F/F_0 values in a subset of wells on a 96 well plate using a checkerboard pattern to estimate Z'. Fluorescence (F) is calculated as the average of 5 data points acquired between 30 - 50 min after the addition of 5 µM ML297, another potent GIRK activator.

Example Results (continued)

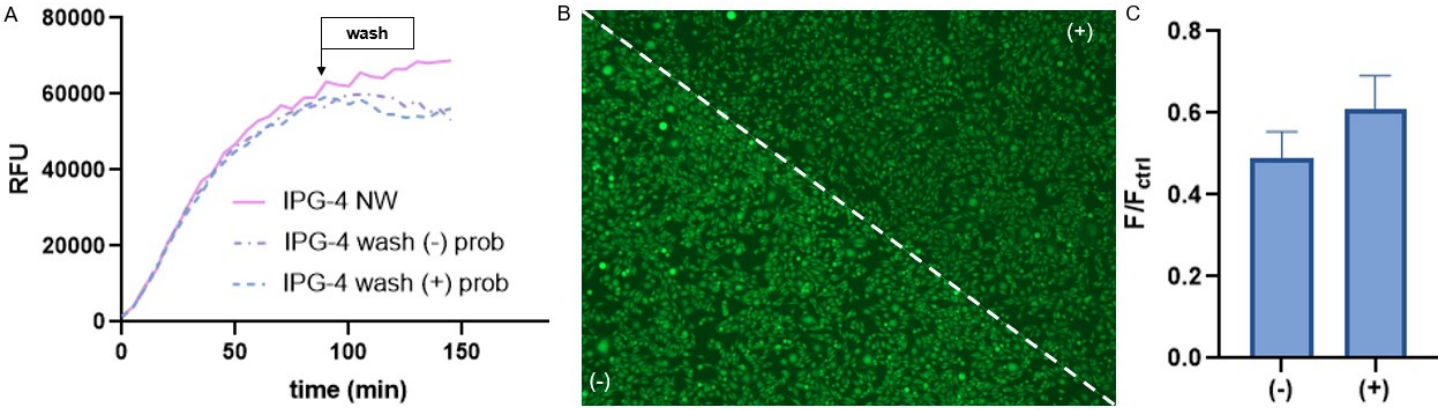

Figure 5. IPG-4 is not a P-gp substrate. A) Kinetic fluorescence profile of IPG-4 loaded CHO GIRK cells during loading and after a wash step was conducted (NW = no wash). Wash solution was composed of HHBSS with 1X TRS and with or without 1X probenecid. Data was acquired using a Cytation 5 plate reader (Ex: 515(9) nm, Em: 545(15) nm). Significant dye efflux was not observed in the presence or absence of probenecid over 1 hour. B) Fluorescence images of IPG-4 loaded, CHO GIRK cells loaded in the absence (-) or presence (+) of probenecid for 90 min. Cells loaded without probenecid are slightly brighter. Images were acquired using GFP filters on a Cytation 5 plate reader. C) Fluorescence ratio of CHO GIRK cells treated with 5 µM ML297 for 30 min versus a vehicle control. Data acquired using a no wash assay conducted without (-) or with (+) probenecid in the dye loading solution. A larger dynamic range is observed in probenecid-free (-) group (error bars represent standard deviation, n = 9 wells).

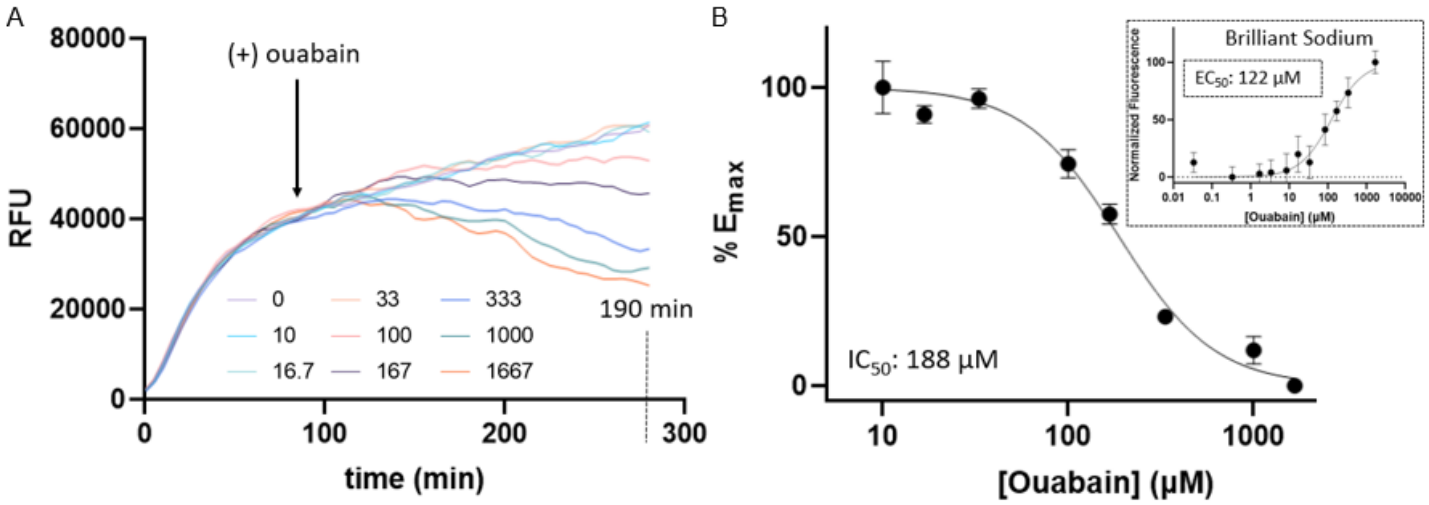

Figure 6. Ouabain dose response in CHO K1 cells measured using IPG-4. A) Kinetic fluorescence plots (n = 3 smoothed curves) of IPG-4 fluorescence during loading (0 - 90 min) and for 190 min after the addition of ouabain. Ouabain inhibits Na^+/K^+-ATPase, reducing intracellular K^+ over time. Reported ouabain concentrations are in µM. Data was acquired using a Cytation 5 plate reader (Ex: 515(9) nm, Em: 545(15) nm). B) Dose response curve using data acquired 190 min after the addition of ouabain. Reported IC_{50} is in line with data obtained using ION's Brilliant Sodium Assay (inset) and literature reported values.