ION BIOSCIENCES

Instructions

IPG-4 TMA^+ SALT

Materials Needed

Name	Volume	Containers	Storage
IPG-4 TMA^+ salt (50 or 500 µg vial)	Dry	1	-20° C
Deionized water (not provided)			
Assay Buffer (not provided)			

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 TMA^+ 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 potassium concentrations in solutions. **Important note:** IPG-4 TMA^+ salt does not dissolve well when buffer is added directly to the vial. Always dissolve the dye in deionized water, before diluting in your buffer of choice.

1. Dissolve IPG-4 TMA^+ salt in **deionized water** (not provided) to the desired stock concentration (e.g. 300 µM).
2. Make a buffer (e.g. HBSS) solution with a high concentration of the monovalent cation (e.g. K^+) to be studied.
3. Make another buffer solution that does not contain the monovalent cation of interest.
4. Dilute the IPG-4 TMA^+ stock concentration to the same working concentration in both buffer solutions (e.g. 3 µM).
5. Place 200 µL of the high cation concentration solution in the first well, column 1. Add 100 µL of the cation-free concentration solution to the rest of the wells in the same row.
6. Take 100 µL of the high cation solution and add it to the 100 µL of cation-free solution in the next well, column 2. Mix evenly, take 100 µL of this solution and add it to the following well in column 3.
7. Repeat these steps until column 11. Once solution for column 11 is mixed evenly, discard 100 µL. Make sure solution in column 12 is only the cation-free solution.
8. Acquire fluorescence read out for the wells using an excitation of ~525 nm and emission of ~545 nm.

Example Results

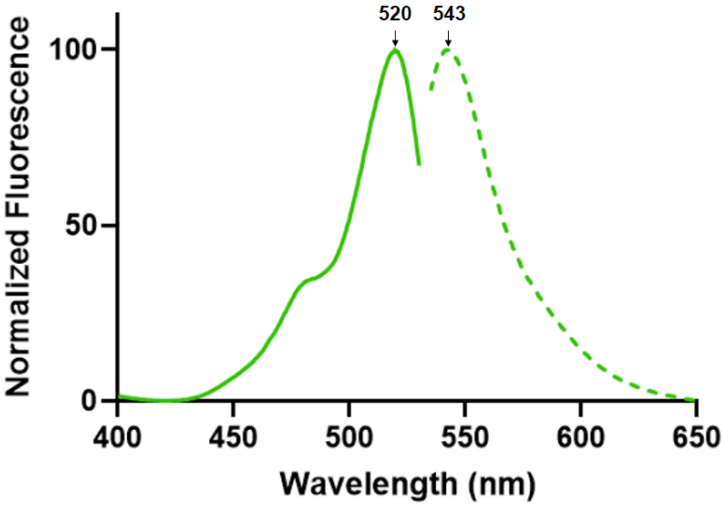

Figure 1. Excitation and emission spectra of IPG-4. Measured in sodium bicarbonate buffer. Maxima are labeled at the top of each curve.

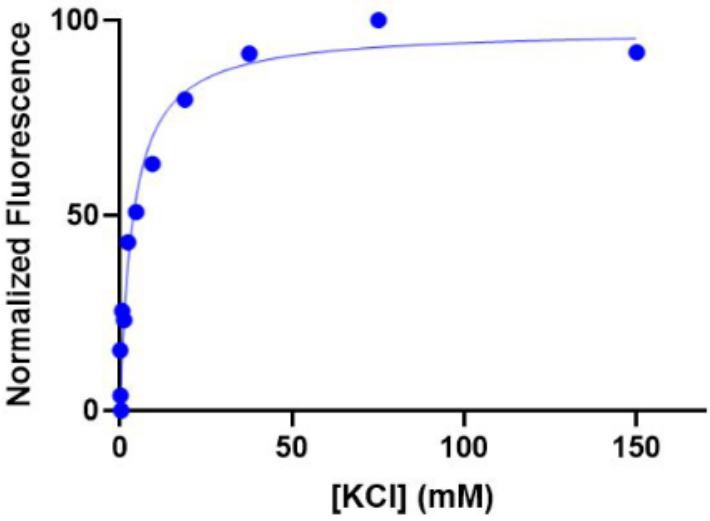

Figure 2. Saturation binding curve. Measured in TRIS buffer containing bovine serum albumin and $MgCl_2$ with TMACl to maintain constant ionic strength = 150 mM. Estimated K_d of ~4 mM.

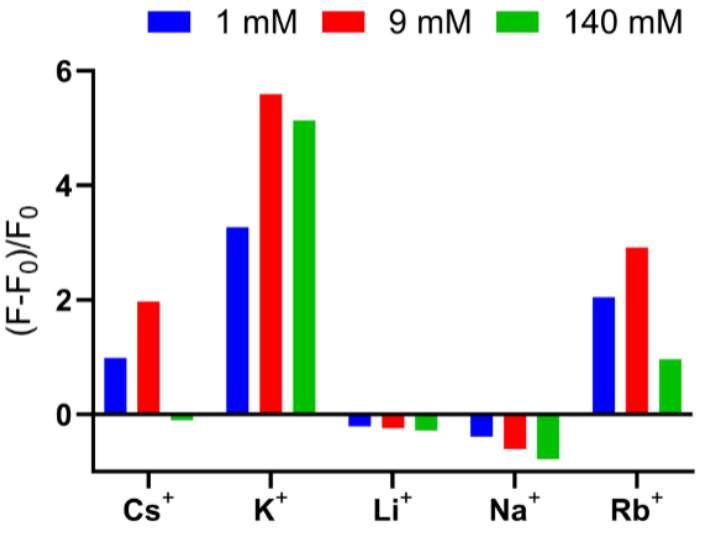

Figure 3. IPG-4 response to monovalent cations. The maximum relative fluorescence intensity Is measured in solutions comprised of 140 mM TMACl, 10 mM TRIS-HCl, 3 µM IPG-4 TMA^+ salt, and varying total monovalent ion concentrations. F_0 represents the fluorescence of an ion-free reference solution.