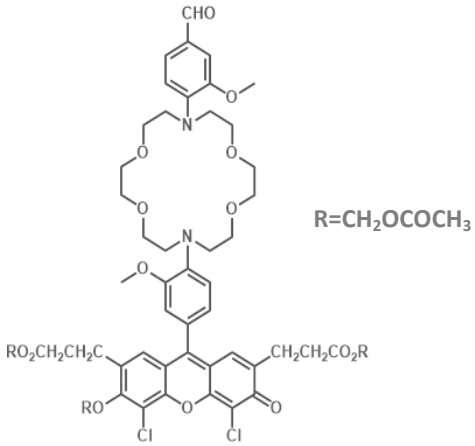
IPG-1 AM

05/21

ALTERNATE NAMES: Potassium (K^+) indicator; IPG-1 membrane permeable; IPG-1 acetoxymethyl ester; Fluorescent Potassium indicator

CATALOG #:
B3140-5PK 5 x 50 µg
B3140-500 500 µg

STRUCTURE:



MOLECULAR WEIGHT: 1140

APPEARANCE: Red-orange powder

PURITY: ≥ 90%

SOLUBILITY: ~2 mg/ml in DMSO

DESCRIPTION: IPG-1 is a yellow-green fluorescent, intracellular potassium (K^+) indicator for measuring changes at most intracellular K^+ concentrations. It is a small, synthetic fluorochrome which incorporates a K^+-binding moiety. When K^+ binds, the quenching is relieved and the fluorescence dramatically increases. IPG-1 has Ex/Em: 525 nm/545 nm and a high-sensitivity to detect small changes in K^+ concentration. IPG-1 has a lower affinity (K_d = 50 mM) than IPG-2 (Cat. Nos. B3142, B3143; K_d = 18 mM) or IPG-4 (Cat. Nos. B3144, B3145; K_d = 7 mM). IPG-1 responds to modest changes in intracellular K^+ concentrations resulting from activation of plasma membrane K^+ channels.

IPG-1 AM is the membrane permeable form of the potasssium indicator (Cat. No. B3141). 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. IPG-1 is compatible with a wide variety of detectors including fluorescent microscopes, plate readers, flow cytometers, and fluorescent indicator-doped solid-state sensors.

STORAGE TEMPERATURE: -20 ºC. 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.
7. Dissolve IPG-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. 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. Recommended volumes are: 35 mm dish or 6-well plate, 1.5 ml; 96-well plate, 100 µl; 384-well plate, 20 µl. 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 ºC 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. Rimmele, T.S., Chatton, J.Y. A Novel Optical Intracellular Imaging Approach for Potassium Dynamics in Astrocytes. *PLOS ONE* 9(10): e109243 (2014).
2. Woo, J., Jang, M.W., Lee, J., et al. The molecular mechanism of synaptic activity-induced astrocytic volume transient. *J Physiol*. 2020 Oct;598(20):4555-4572 (2020).

RELATED PRODUCTS:

FURA-5F/AM (Cat. No. 9551)
ING-2 AM (Cat. No. B3137)
FURA-2 Am (Cat. No. 2243)
ING-2 TMA+ Salt (Cat. No. B3138)
FURA-4F/AM (Cat. No. 9550)

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