Protocol Booklet

Product Code(s)	HB6781
Product Name(s)	IPG-4 AM
Purpose	Measurement of intracellular K^+ in cultured cells

Please note: This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use

Contents

Product Overview

IPG-4 AM is a membrane permeable potassium indicator (Excitation 525nm, Emission 545nm) which is compatible with a wide variety of detectors (e.g. fluorescent microscopes, plate readers, flow cytometers, and fluorescent indicator-doped solid-state sensors). It can be used with common filter sets (e.g. YFP and FITC) and multiphoton approaches. Higher affinity for potassium (K_d = 7mM) compared to IPG-2 (K_d = 18mM) Suitable for diverse applications such as extracellular K^+ sensing and monitoring intracellular K^+ dynamics. Synthetic fluorochrome which incorporates a K^+-binding moiety. Under conditions where K^+ is not bound, the fluorescence of the sensor is significantly quenched. When K^+ is bound, the quenching is relieved, and the fluorescence of the sensor dramatically increases. Additionally, IPG-4 AM is not a MDR1 (pgp) substrate therefore is compatible with probenecid free assays.

Components & Storage

ING-4 AM is provided as:

SKU	Component	Quantity	Storage Temperature
HB6781	IPG-4 AM	500µg	-20°C

This protocol additionally requires:

Component	Quantity	Storage Temperature
DMSO	25µl	RT
Pluronic F-127	10mg	4°C
Assay Buffer (HEPES-buffered Hank's Balanced Salt Solution (pH = 7.3)*	10ml	RT

* Please see recipe at the end of this protocol book.

Protocol

The following protocol provides general guidelines for using IPG-4 AM to measure intracellular potassium in cultured cells. All loading conditions (dye concentration, temperature, and time) should be optimized for your specific assay, application, and instrumentation.

1. Culture cells following standard protocols to approximately 80-100% confluence.
2. Prepare the loading solution freshly following the below table, vortex well and use within 2 hours.
3. Remove the cell culture medium, briefly wash in plain media (without serum), then add dye loading solution. Recommend volumes are:
    a. 35mm dish / 6-well plate - 1.5 mL/well,
    b. 96 well plate - 100 µL/well,
    c. 384 well plate - 20 µL/well,
4. Incubate in a cell culture incubator at 37ºC for 60-90 minutes.
5. Read fluorescence using a plate reader (Excitation: 525nm, Emission 545nm) or image using a fluorescence microscope using a compatible filter set (e.g. FITC, GFP, YFP).
6. Add test compounds to cells.
7. Measure fluorescence for up to 1hr post compound addition. It is recommended to measure in 5 minute increments and optimise for the experimental design.

Recipes

IPG-4 Loading Solution

Component	Concentration	Quantity	Notes
IPG-4 AM	3.75µM	50µg	Dissolve in DMSO then aliquot and store any unused dye at -20°C
Assay Buffer	1X	10ml	Normally HEPES buffered HBSS but other buffers have been also successfully used.
Pluronic F-127	0.1%	10mg	Surfactant that helps the dissolution of dye therefore ensuring even dye distribution and cellular loading.

Please note: Combine components then vortex thoroughly. Use within 2 hours of creation. Do not freeze.

HEPES-buffered Hank's Balanced Salt Solution (Assay Buffer)

Component	MW (g/mol)	g/L	Concentration (mM)
Calcium Chloride	110.98	0.14	1.26
Magnesium Chloride Hexahydrate	203.30	0.1	0.49
Magnesium Sulfate Heptahydrate	246.47	0.1	0.41
Potassium Chloride	74.55	0.4	5.33
Potassium Phosphate Monobasic	136.09	0.06	0.44
Sodium Bicarbonate	84.01	0.35	4.17
Sodium Chloride	58.44	8	138.00
Sodium Phosphate Dibasic	141.96	0.048	0.34
D-Glucose (Dextrose)	180.16	1	5.56
HEPES	238.30	4.76	20.00

Please note: Add all components to dH_2O, mix well then adjust to pH 7.3

Guidelines, precautions, troubleshooting

Please contact our technical support team at technicalhelp@hellobio.com for advice on how to resolve any problems encountered when using this product. Observe safe laboratory practice and consult the safety datasheet. Please see the datasheet on our website for general guidelines, precautions, limitations on the use of the product.

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