



Protocol Booklet

Product Code(s)	HB18045
Product Name(s)	IPG-2 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	3
Components & Storage	3
Protocol	3
Recipes	4
IPG-2 Loading Solution	4
HEPES-buffered Hank's Balanced Salt Solution (Assay Buffer)	4
Guidelines, precautions, troubleshooting	4
Contact	4
For customers in the UK, Europe and Rest of the World	4
For customers in the USA, Canada and South America	4



Product Overview

IPG-2 AM is a yellow-green fluorescent potassium indicator ($K_d = 18\text{mM}$) which can be used with common filter sets (e.g. YFP and FITC) and multiphoton approaches (Excitation 525nm, Emission 545nm). It is 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. Compatible with a wide variety of detectors including fluorescent microscopes, plate readers, flow cytometers, and fluorescent indicator-doped solid-state sensors.

Components & Storage

ING-2 AM is provided as:

SKU	Component	Quantity	Storage Temperature
HB18045	IPG-2 AM	500 μg	-20°C

This protocol additionally requires:

Component	Quantity	Storage Temperature
DMSO	25 μl	RT
Pluronic F-127	10mg	4°C
Probenecid	7.7mg	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-2 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 37°C for 60 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).



Recipes

IPG-2 Loading Solution

Component	Concentration	Quantity	Notes
IPG-2 AM	4.4µM	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.
Probenecid	2.7mM	7.7mg	Anion transport inhibitor that improves intracellular dye retention. Not required for all cell types, it is recommended in most cases to optimize assay performance.

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 dH₂O, mix well then adjust to pH 7.3

Guidelines, precautions, troubleshooting

Please contact our technical support team at technicalhelp@helloworld.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.

Contact

For customers in the UK, Europe and Rest of the World

Customer Care customercare@helloworld.com

Technical support technicalhelp@helloworld.com

By telephone: +44(0)117 318 0505

By fax: +44(0)117 981 1601

Opening hours: 8.30 am - 5.00 pm GMT weekdays

For customers in the USA, Canada and South America

Customer Care customercare-usa@helloworld.com

Technical support technicalhelp@helloworld.com

By telephone: +1-609-683-7500

By fax: +1-609-228-4994

Opening hours: 9.00 am - 5.00 pm EST weekdays