IPG-4 AM

Table 1 - Materials Needed	Most Items Available from ION Biosciences	
Name	**Volume**	**Description/Purpose**
IPG-4 AM (50 µg vial)	Dry - 1 Vial	Membrane Permeable Potassium Dye Indicator
DMSO[1]	25 µL	Solvent for Dissolution of Dye
100X Pluronic F-127 solution[2]	100 µL	Biocompatible Surfactant for Dye Loading
50X TRS solution (optional)[3]	200 µL	Extracellular Fluorescence Masking Agent
Assay Buffer - We Suggest One of the Following:		
⇒ 1X HEPES-Buffered Hank's Balanced Salt Solution	10 mL	1X - Ready to Use Assay Buffer
⇒ 10X Brilliant Thallium Assay Buffer	1 mL	10X - Concentrated Assay Buffer
Water	As Needed	Dilution of 10X Assay Buffer (if used)

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.

Laboratory Procedures

The following protocol provides general guidelines for using this dye to measure intracellular potassium. 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 25 µL DMSO[1] to the tube containing IPG-4 AM.
3. Vortex until IPG-4 AM is fully dissolved. Centrifuge briefly to collect all contents at the tube bottom.
4. Add the appropriate volume (see **Table 2**, next page) of 1X Assay Buffer to a 15 mL conical tube. If using 10X Assay Buffer, add the appropriate volume of Water (**Table 2**) and 1 mL of 10X Assay Buffer to a 15 mL conical tube.
5. Add 100 µL of 100X Pluronic F-127[2] solution to the conical tube from **step 4**.
6. (Optional) Add 200 µL of 50X TRS[3] solution to the conical tube from **step 5**.
6. Vortex conical tube from **step 6** briefly to mix.

Procedure Continues on Next Page

Laboratory Procedures (continued)

8. Add the entire contents of the IPG-4 AM in DMSO solution from **step 3** to the conical tube from **step 7** to make the **Dye Loading Solution**[4].
9. Vortex the **Dye Loading Solution**[4] from **step 8** briefly to mix.

Table 2 Dye Loading Solution[4]	1X Assay Buffer		10X Assay Buffer	
Name	**Method A**	**Method B**	**Method C**	**Method D**
IPG-4 AM in DMSO[1] Solution	25 µL	25 µL	25 µL	25 µL
100X Pluronic F-127[2] solution	100 µL	100 µL	100 µL	100 µL
50X TRS[3] solution	200 µL	-	200 µL	-
1X HEPES-Buffered Hanks Balanced Salt Solution	9.7 mL	9.9 mL	-	-
10X Brilliant Thallium Assay Buffer	-	-	1 mL	1 mL
Water	-	-	8.7 mL	8.9 mL
Total	10 mL	10 mL	10 mL	10 mL

10. Remove the cell culture medium and add the **Dye Loading Solution**[4] from **step 9**. Recommend volumes are: 35 mm dish or 6-well plate, 1.5 mL; 96-well plate, 100 µL; 384-well plate, 20 µL.[5]
11. Incubate in a cell culture incubator at 37ºC for 60-90 minutes.
12. Conduct a wash[5] step to remove the **Dye Loading Solution**[4] and replace with cell culture medium or assay buffer. Repeat, if necessary, to completely remove extracellular dye[6].
13. Read fluorescence using a plate reader (Ex/Em: 515 nm/545 nm)[7] or image using a fluorescence microscope (using filters for YFP, GFP or FITC).[6]
14. Add compounds of interest to cell-containing well plate at desired concentrations.
15. Reacquire fluorescence data using the same instrument used in **step 13**. This step can be performed up to 1 hour after compound addition. 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.

Table 3 Recommended Instrument Settings	
Setting	**Recommendation**
Read Mode (Plate Readers)	'Bottom' read mode only
Ex/Em wavelengths[7]	~515 nm/545 nm
Cutoff wavelength	530 nm
Filter selection	GFP, FITC, YFP
Contact support@ionbiosciences.com for additional recommendations and guidance on optimizing to your application.	

Laboratory Procedures - Footnotes

[1] DMSO is hygroscopic and should be stored tightly closed. Wet solvent causes difficulties with dissolution of the dye.

[2] Pluronic F-127 is a biocompatible surfactant that aids in dye dissolution, ensuring equitable dye distribution and cellular loading.

[3] TRS is a membrane impermeant dye useful for masking extracellular fluorescence. Caution is advised when using TRS or any other extracellular masking solutions as they may have undesirable effects on assay performance for the target of interest.

[4] The Dye Loading Solution should be used within 2 hours of dye addition for best results.

[5] 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 100X Pluronic F-127 and 50X TRS in your dye loading buffer.

[6] To minimize extracellular background, dye loading solution can be replaced with assay buffer containing 1X TRS solution (optional).

[7] To prevent bleed-through or spectral overlap, the Ex/Em wavelengths may need to be optimized by broadening the interval between the wavelengths.

Additional Information

Dye indicator and buffer reagents can be purchased either directly from our website or by contacting our Sales Department. Custom and bulk sizes are also available. Contact Sales for more information.

Table 4	Available Reagents	Available Sizes	
Name		Size	Catalog #
IPG-4 AM		500 µg x 1 Vial	3021C
		50 µg x 20 Vials	3021F
		50 µg x 5 Vials	3021G
100X Pluronic F-127 Solution		10 mL Bottle	7601A
50X TRS Solution		20 mL Bottle	7060A
1X HEPES-Buffered Hank's Balanced Salt Solution (1X HHBSS)		100 mL Bottle	7001
10X Brilliant Thallium Assay Buffer		10 mL Bottle	7010T