ION Lithium Green-1 (ILG-1) AM

Table 1 - Materials Needed	Most Items Available from ION Biosciences	
Name	**Volume**	**Description/Purpose**
ILG-1 AM (50 µg vial)	Dry - 1 Vial	Membrane Permeable Lithium Dye Indicator
DMSO[1]	25 µL	Solvent for Dissolution of Dye
100X Pluronic F-127 solution[2]	100 µL	Biocompatible Surfactant for Dye Loading
100X Probenecid solution (optional)[3]	100 µL	Intracellular Dye Retention Aid
50X TRS (optional)[4]	200 µL	Extracellular Fluorescence Masking Agent
10X Brilliant Lithium Assay Buffer	1 mL	10X - Concentrated Assay Buffer
10X Sodium Stimulus Buffer	0.5 - 1 mL	Stimulus Component $[Na^+]$=1.38 M $[Li^+]$=0 M
10X Lithium Stimulus Buffer	0.5 mL	Stimulus Component $[Na^+]$=0 M $[Li^+]$=1.38 M
$Na_V1.X$ Inhibitors and or Activators	As Needed	$Na_V1.X$ Channel Modulators
Water	As Needed	Dilution of 10X Buffer and Stimulus Solutions

Description

ION Lithium Green 1 AM (ILG-1 AM), is a membrane-permeable, lithium-sensitive fluorescent dye that enables solution and cell-based lithium detection. Due to its high affinity and selectivity for lithium ions, ILG-1 enables rapid determination of lithium concentrations in a variety of samples compared to the other commercial lithium assays.

As a key component in our Brilliant Lithium Assay, ILG-1 AM is also compatible with intracellular lithium detection and can be used for functional $Na_V1.X$ channel screening where lithium is a surrogate ion for sodium. Lithium readily permeates open $Na_V1.X$ channels while bypassing many endogenous sodium-handling mechanisms, resulting in a robust and selective readout of channel activity. Upon depolarization or pharmacological activation of $Na_V1.X$ channels, lithium accumulates intracellularly producing a rapid, quantifiable fluorescence increase that reflects real time channel activity.

General Considerations

The following protocol provides general guidelines for using this dye to measure intracellular lithium flux. All loading conditions (dye concentration, temperature, and time) should be optimized for your specific assay, cell type, and application. ***Performance of ILG-1 AM is highly dependent on the assay buffer used.*** ION's 10X Brilliant Lithium Assay Buffer is formulated to maximize the performance of ILG-1 AM, and equivalent performance may not be attained without the use of this assay buffer. Purchasing our Brilliant Lithium Assay Kit (Cat# 9200) is strongly recommended as this includes all the components necessary to obtain the best possible performance of ILG-1 AM. 10X Brilliant Lithium Assay Buffer is not a regular catalog item but may be purchased by contacting our Sales department.

Laboratory Procedures

1. Allow all reagents to warm to room temperature before proceeding.
2. Add 25 µL DMSO[1] to the tube containing ILG-1 AM.
3. Vortex until ILG-1 AM is fully dissolved. Centrifuge briefly to collect all contents at the tube bottom.
4. Add 1 mL 10X Brilliant Lithium Assay Buffer and the appropriate volume of Water (see **Table 2**, below) 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 100 µL of 100X Probenecid[3] solution to the conical tube from **step 5**.
7. (Optional) Add 200 µL of 50X TRS[4] solution to the conical tube from **step 6**.
8. Vortex conical tube from **step 7** briefly to mix.
9. Add the entire contents of the ILG-1 AM in DMSO solution from **step 3** to the conical tube from **step 8** to make the **Dye Loading Solution**[5].
10. Vortex the **Dye Loading Solution**[5] from **step 9** briefly to mix.

Table 2 Dye Loading Solution[5]	**Example Formulations**			
Name	**Method A**	**Method B**	**Method C**	**Method D**
ILG-1 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
100X Probenecid[3] solution (optional)	100 µL	100 µL	-	-
50X TRS[4] (optional)	200 µL	-	200 µL	-
10X Brilliant Lithium Assay Buffer	1 mL	1 mL	1 mL	1 mL
Water	8.6 mL	8.8 mL	8.7 mL	8.9 mL
Total	**10 mL**	**10 mL**	**10 mL**	**10 mL**

11. (Optional) Prepare a **Wash Solution**[6] in a 15 mL centrifuge tube by adding the appropriate amounts of water and 10X Brilliant Lithium Assay Buffer as shown in **Table 3** (next page). Briefly vortex to mix well.
12. Completely remove the cell-culture medium from the 96-well or 384-well microplate containing the cells of interest
13. Wash the cell-containing microplate once with **Wash Solution**[6]. 100 µL/well for 96-well plate and 20 µL/well for 384-well plate. Completely remove the **Wash Solution**[6] from the 96-well or 384-well plates.

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Laboratory Procedures (Continued)

Table 3 Wash Solution[6] (Optional)	
Name	**Volume**
10X Brilliant Lithium Assay Buffer	1 mL
Water	9 mL
Total	**10 mL**

14. Add the **Dye Loading Solution** from **step 10** to the cell-containing microplate. 100 µL/well for 96-well plate and 20 µL/well for a 384-well plate.

15. Incubate the cell-containing microplate with the **Dye Loading Solution** for 60 minutes at 37°C.

Note: If screening for $Na_V1.X$ Inhibitors, continue with steps 16–18; otherwise, proceed to Step 19.

16. (Optional) Prepare an Inhibitor Solution in a 15 mL centrifuge tube by adding the appropriate amounts of water, 10X Brilliant Lithium Assay Buffer , and 3X concentrated $Na_V1.X$ inhibitors as shown in **Table 4** below. Briefly vortex to mix well.

17. Add the **Inhibitor Solution** from **step 15** to the cell-containing microplate. 50 µL/well for a 96-well plate and 10 µL/well for a 384-well plate.

18. Incubate the cell-containing microplate for 15 minutes at 37°C.

Table 4 Inhibitor Solution (Optional)	
Name	**Volume**
10X Brilliant Lithium Assay Buffer	1 mL
$Na_V1.X$ Inhibitors	Enough for 3X concentration at total volume
Water	Q.S. to total volume
Total	**10 mL**

19. Prepare a **Stimulus Solution**[7] containing Li^+ and Na^+ ions along with $Na_V1.X$ activators. The concentration of lithium ions in the **Stimulus Solution**[7] should be optimized for each assay. We recommend a final concentration of 5 mM Li^+ in the wells as a starting point. **Table 5** (next page) shows several examples of **Stimulus Solutions**[7] using the 10X Sodium Stimulus Buffer and 10X Lithium Stimulus Buffers as supplied in our Brilliant Lithium Assay Kits. Prepare the **Stimulus Solution**[7] in a 15 mL centrifuge tube by adding the appropriate amounts of water, 10X Sodium Stimulus Buffer, 10X Lithium Stimulus Buffer and 4X concentrated $Na_V1.X$ activators as shown in **Table 5** (next page). Briefly vortex the **Stimulus Solution**[7] to mix well.

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Laboratory Procedures (continued)

Table 5 Stimulus Solution[7]			
Name	**Example A**	**Example B**	**Example C**
10X Sodium Stimulus Buffer ([Li^+] = 0 M, [Na^+] = 1.38 M)	800 µL	900 µL	950 µL
10X Lithium Stimulus Buffer ([Li^+] = 1.38 M, [Na^+] = 0 M)	200 µL	100 µL	50 µL
$Na_V1.X$ Activators	Enough for 4X concentration at total volume		
Water	Q.S. to total volume		
Total	**10 mL**	**10 mL**	**10 mL**

20. Load the **Stimulus Solution**[7] from **step 19** into a V-shaped reagent plate for assay readout. 100 µL/well for a 96-well plate and 20 µL/well for a 384-well plate.
21. Transfer the cell-containing microplate and reagent plate to a kinetic-imaging plate reader (e.g. WaveFront Panoptic, Hamamatsu FDSS, Molecular Devices FLIPR, or Molecular Devices FlexStation).
22. Acquire data using an excitation wavelength of ~515 nm, an emission wavelength of ~545 nm, and an acquisition frequency of ~1 Hz. Excitation sources commonly used for fluorescein, Fluo-4, and GFP (480-490 nm) are compatible with ION's ILG-1 AM fluorescent dye. See **Table 6** below for recommended instrument settings.
23. Begin baseline data acquisition for 30 seconds, then transfer the volume from the reagent plate to the cell-containing microplate. 50 µL/well for a 96-well plate and 10 µL/well for a 384-well plate. Continue data acquisition for an additional 270 seconds or longer.

Table 6 Recommended Instrument Settings	
Setting	**Recommendation**
Read Mode	'Bottom' read mode only
Ex/Em wavelengths[8]	~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] 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.

[4] 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.

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

[6] 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, 100X Probenecid, and 50X TRS in your dye loading buffer.

[7] The timing and volume of stimulus solution addition may vary. Some experiments may include the addition of other solutions to the cell-containing microplate prior to the addition of the stimulus solution. In these cases, the volume of the stimulus solution addition should be altered to account for the additional volume of solution in the cell-containing microplate.

[8] 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

Name	Available Sizes	
	Size	**Catalog #**
ILG-1 AM	500 µg x 1 Vial	2215C
	50 µg x 10 Vials	2215F
	50 µg x 3 Vials	2215G
100X Pluronic F-127 Solution	10 mL Bottle	7601A
100X Probenecid Solution	10 mL Bottle	7300P-100
50X TRS Solution	20 mL Bottle	7060A
10X Brilliant Lithium Assay Buffer	Not a Regular Catalog Item. Contact Sales About Purchase.	
10X Sodium Stimulus Buffer [Na^+]=1.38 M [Li^+]=0 M	Not a Regular Catalog Item. Contact Sales About Purchase.	
10X Lithium Stimulus Buffer [Na^+]=0 M [Li^+]=1.38 M	Not a Regular Catalog Item. Contact Sales About Purchase.	