Brilliant Lithium Assay

Catalog No. 9200

Introduction

Voltage-gated sodium channels ($Na_V1.X$) are essential membrane proteins that initiate and propagate action potentials by mediating rapid sodium influx in response to membrane depolarization. They underlie key physiological processes including neuronal signaling ($Na_V1.1$, $Na_V1.2$, $Na_V1.6$), cardiac conduction ($Na_V1.5$), and pain sensation ($Na_V1.7$ –$Na_V1.9$). Abnormal $Na_V1.X$ activity is implicated in disorders such as epilepsy, cardiac arrhythmias, and chronic pain, positioning these channels as high-value therapeutic targets. Conventional methods for assessing $Na_V1.X$ function such as patch-clamp electrophysiology and radiolabeled ion flux assays are often low-throughput, technically demanding, and reliant on specialized instrumentation.

ION Biosciences' Brilliant Lithium Assay overcomes these limitations with a next-generation, fluorescence-based approach for high-throughput screening of $Na_V1.X$ channel modulators. The assay uses ION Lithium Green 1 AM (ILG-1 AM), a membrane-permeable, lithium-sensitive fluorescent dye that enables functional $Na_V1.X$ channel measurements using lithium as a surrogate ion. 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.

Compared to the Brilliant Sodium 2, Brilliant Lithium delivers approximately a two-fold larger screening window, enhanced assay sensitivity, and larger dynamic range. This improved performance supports reliable EC_{50} and IC_{50} determination in both 96- and 384-well high-throughput screening formats.

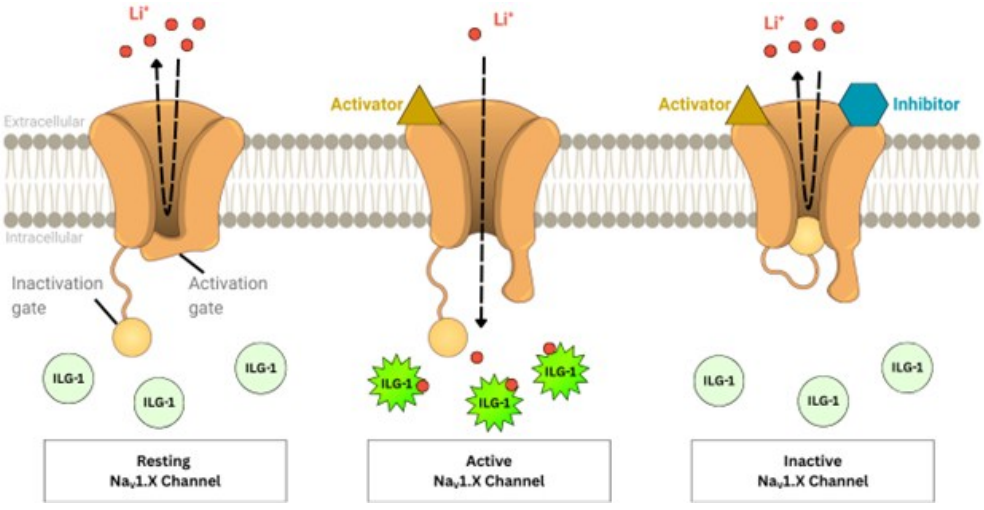

Figure 1. Brilliant Lithium Assay Principles. Cells expressing voltage-gated sodium channels ($Na_V1.X$) are first incubated with a dye-loading solution containing ILG-1, a fluorescent indicator sensitive to lithium ions. Lithium ions act as a surrogate for sodium ions, readily passing through open $Na_V1.X$ channels to provide a functional readout of channel activity. Addition of a stimulus solution containing $Na_V1.X$ channel activators (e.g., veratridine, deltamethrin) together with lithium ions induces channel opening and enables rapid intracellular accumulation of lithium. The resulting increase in intracellular lithium is detected as an increase in fluorescence, providing a direct, real-time measurement of $Na_V1.X$ channel activity. Treatment with sodium channel inhibitors (e.g., tetracaine, TTX, suzetrigine) attenuates activator-induced lithium influx, resulting in a reduced fluorescence response.

Storage and Stability

Upon receipt, store components at the temperatures indicated on each label. Kit components are stable[1] for up to 12 months from date of shipment when stored as directed.

Table 1	Kit Contents	*Flex* Kit Cat# 9200-10		*pIONeer* Kit Cat# 9200-2		
Label	**Name**	**Size**	**Qty**	**Size**	**Qty**	**Storage**
Reagent A	ILG-1 AM	50 µg Vial	10	50 µg Vial	2	-20° C
Reagent B	DMSO[1]	225 µL Vial	1	Not included in pIONeer		-20° C
Reagent C	50X DySolv	4 mL Bottle	1	800 µL Vial	1	4° C
Reagent D	10X Brilliant Lithium Assay Buffer	30 mL Bottle	1	6 mL Bottle	1	4° C
Reagent E	50X TRS[2]	4 mL Bottle	1	800 µL Vial	1	4° C
Reagent F	50X Probenecid[2]	4 mL Bottle	1	800 µL Vial	1	4° C
Reagent G	10X Sodium Stimulus Buffer $[Na^+]$ = 1.38 M, $[Li^+]$ = 0 M	10 mL Bottle	1	2 mL Bottle	1	4° C
Reagent H	10X Lithium Stimulus Buffer $[Na^+]$ = 0 M, $[Li^+]$ = 1.38 M	4 mL Bottle	1	800 µL Vial	1	4° C

Getting Started

Before beginning your experiment, ensure you have all the necessary reagents and materials. ION Biosciences' Brilliant Lithium Assay includes everything required for the assay, except for $Na_V1.X$ modulators and $Na_V1.X$-expressing cell lines. A fluorescence plate reader with excitation capabilities between 485–525 nm and emission collection around 545 nm is also needed; ideally, the reader should support kinetic data collection at about 1 Hz. Examples of suitable instruments include the WaveFront Panoptic, Hamamatsu FDSS, Molecular Devices FLIPR, and Molecular Devices FlexStation.

These instructions are designed for both 96-well and 384-well microplate formats. Adjustments may be needed for other formats, such as 1536-well plates or non-adherent cells. ILG-1 AM should be protected from direct light and solutions containing ILG-1 AM should be used within 2 hours of preparation. We recommend seeding $Na_V1.X$-expressing cells in 96-well or 384-well plates 24 hours prior to the assay, with 20K cells per well for 96-well plates and 10K cells per well for 384 -well plates.

Laboratory Procedures

1. Add 20 µL of DMSO (**Reagent B**) to the tube containing ILG-1 AM (**Reagent A**).
2. Vortex until Reagent A is fully dissolved.
3. Add appropriate volume of water (**Table 2**, next page) to a 15 mL centrifuge tube.
4. Add 1 mL of 10X Brilliant Lithium Assay Buffer (**Reagent D**) to the tube from **step 3**.

Procedure Continues on Next Page

Laboratory Procedures (Continued)

5. Add 200 µL of 50X DySolv (**Reagent C**) to the tube from **step 4**.
6. Add 200 µL of 50X TRS[2] (**Reagent E**) to the tube from **step 5**.
7. If desired add 200 µL of 50X Probenecid[2] (**Reagent F**) to the tube from **step 6**.
8. Add 20 µL of ILG-1 AM Solution from **step 2** to the tube from **step 7**.
9. Briefly vortex the **Dye Loading Solution** from **step 8** to mix well.

Table 2	**Dye Loading Solution**		
Label	**Name**	**Method A**	**Method B**
Reagent A+B	ILG-1 AM solution in DMSO	20 µL	20 µL
Reagent C	50X DySolv	200 µL	200 µL
Reagent D	10X Brilliant Lithium Assay Buffer	1 mL	1 mL
Reagent E	50X TRS[2]	200 µL	200 µL
Reagent F	50X Probenecid[2]	200 µL	-
	Water	8.4 mL	8.6 mL
	Total	**10 mL**	**10 mL**

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

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

13. Add the **Dye Loading Solution** from **step 9** to the cell-containing microplate. 100 µL/well for 96-well plate and 20 µL/well for a 384-well plate.
14. Incubate the cell-containing microplate with the **Dye Loading Solution** for 60 minutes at 37°C.

Procedure Continues on Next Page

Laboratory Procedures (Continued)

Note: If screening for $Na_V1.X$ Inhibitors, continue with steps 15–17; otherwise, proceed to Step 18.

15. Prepare the Inhibitor Solution in a 15 mL centrifuge tube by adding the appropriate amounts of water, 10X Brilliant Lithium Assay Buffer (**Reagent D**), and 3X concentrated $Na_V1.X$ inhibitors as shown in **Table 4** below. Briefly vortex to mix well.
16. 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.
17. Incubate the cell-containing microplate for 15 minutes at 37°C.

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

18. Prepare the **Stimulus Solution**[4] in a 15 mL centrifuge tube by adding the appropriate amounts of water, 10X Brilliant Sodium Stimulus Buffer (**Reagent G**), 10X Lithium Stimulus Buffer (**Reagent H**) and 4X concentrated $Na_V1.X$ activators as shown in **Table 5** below. Briefly vortex the **Stimulus Solution**[4] to mix well.

Note: The concentration of lithium ions in the Stimulus Solution[4] should be optimized for each assay. A final concentration of 5 mM Li^+ in the wells is the recommended starting point.

19. Load the **Stimulus Solution**[4] from **step 18** 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.

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

Procedure Continues on Next Page

Laboratory Procedures (Continued)

20. 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).
21. 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.
22. 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[5]	~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 at -20° C with desiccant pack to prevent the solvent from becoming wet. Wet solvent causes difficulties with dissolution of the dye. Use the DMSO within 6 months of receipt.

[2] Caution is advised when using Probenecid and/or TRS as they may have undesirable effects on assay performance for the target of interest. Probenecid may be included in the Dye Loading Solution to aid in dye retention. This may be particularly important in certain cell lines (e.g. CHO cells). TRS contains a membrane-impermeant dye useful for masking extracellular fluorescence.

[3] We recommend washing the cells with wash solution only if residual culture medium remains in the well.

[4] 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.

[5] To prevent bleed-through or spectral overlap, the Ex/Em wavelengths may need to be optimized by broadening the interval between the wavelengths. Results displayed were generated on a Wavefront Panoptic.

Data Analysis

1. Plot the relative fluorescence units (F) over time as the raw kinetic fluorescence data (**Figure 2**).
2. Define the initial 30 seconds of relative fluorescence units (F) as the baseline. Average the baseline and define it as F_0. Calculate the ratio of F/F_0 at each time point. Plot F/F_0 over time as the baseline-normalized kinetic fluorescence data (**Figure 3**).
3. Use GraphPad® Prism (or another similarly capable graphing and statistical analysis software) to apply a linear regression to the first 30 seconds after stimulus addition on the baseline-normalized kinetic fluorescence data. Plot the slope or maximum velocity (V_{max}) of the linear regression against the $Na_V1.X$ activator or inhibitor concentration to make a concentration response curve (**Figure 4**, next page).
4. Using GraphPad® Prism, run the non-linear regression "agonist vs response" on the concentration-response curve (V_{max}) to calculate the half-maximal effective concentration (EC_{50}). Alternatively, run the non-linear regression "inhibitor vs response" to calculate the half-maximal inhibitory concentration (IC_{50}) (**Figure 5**, next page).

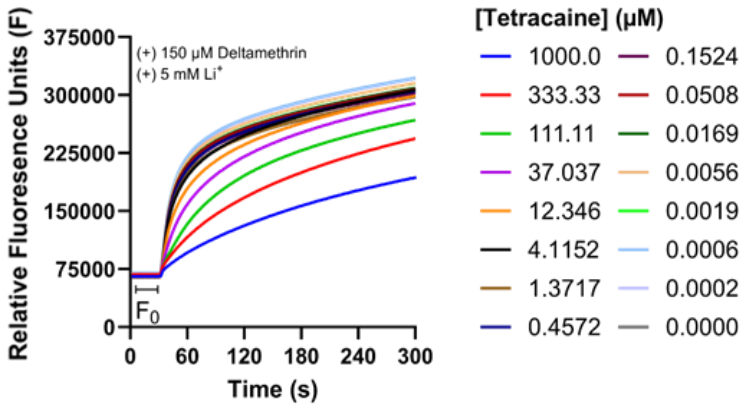

Figure 2. Raw Kinetic Data. Raw kinetic data of $Na_V1.8/\beta1$-expressing HEK293T cells were collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). Relative Fluorescence Units (RFU) were collected for 30 seconds before addition of 5 mM Li^+, 150 µM Deltamethrin, and a dose response of tetracaine . After addition, the plate continued to be read for an additional 270 seconds.

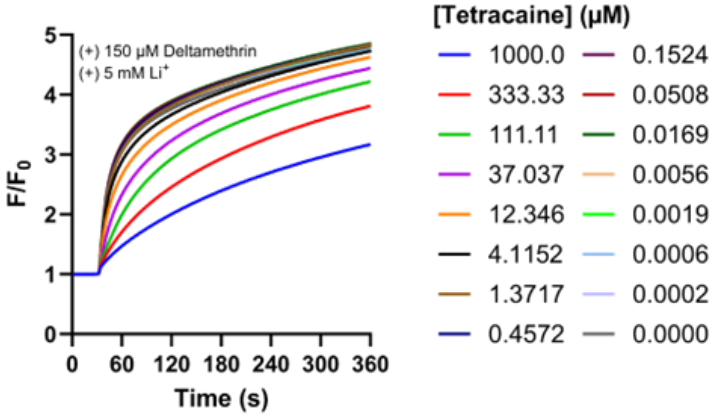

Figure 3. Baseline-Normalized Kinetic Data. Based on the raw kinetic data from figure 2, average the relative fluorescence units (F) of the initial 30 seconds before stimulus addition and define it as the baseline (F_0). Calculate the ratio F/F_0 for each data set and plot F/F_0 over time as the baseline-normalized kinetic data.

Data Analysis (Continued)

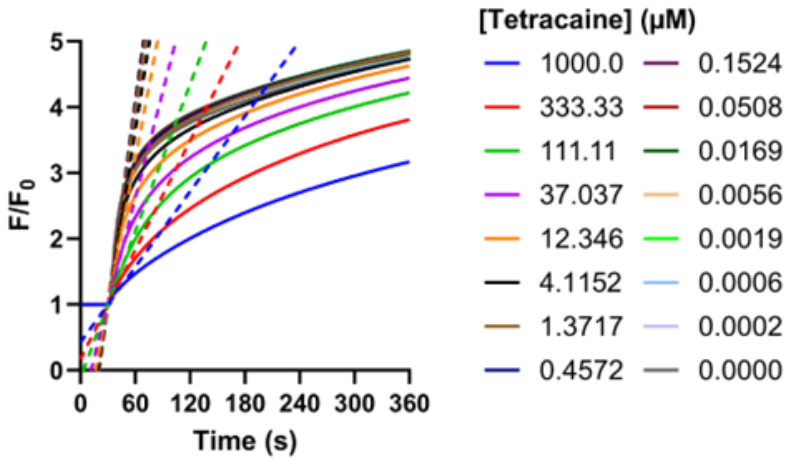

Figure 4. Maximum Velocity (V_{max}) Calculation. Based on the Baseline-Normalized Kinetic Data from **Figure 3**, conduct a linear regression on the first 30 seconds after stimulus addition. Calculate the slope of each data set and define it as the V_{max}.

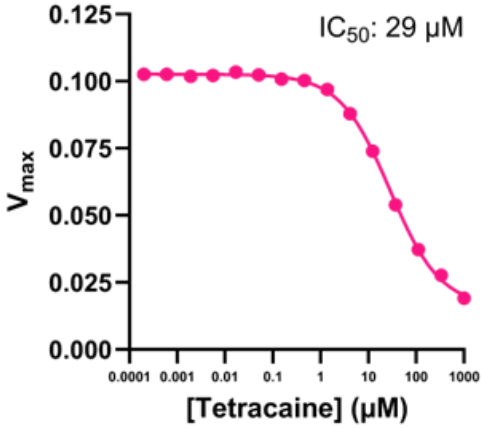

Figure 5. EC_{50} Calculation. Plot the V_{max} or the AUC against the compound concentrations and run the non-linear regression "agonist vs response" in GraphPad® Prism to calculate an EC_{50}. Alternatively, run the non-linear regression "inhibitor vs response" to calculate an IC_{50}.

Example Results

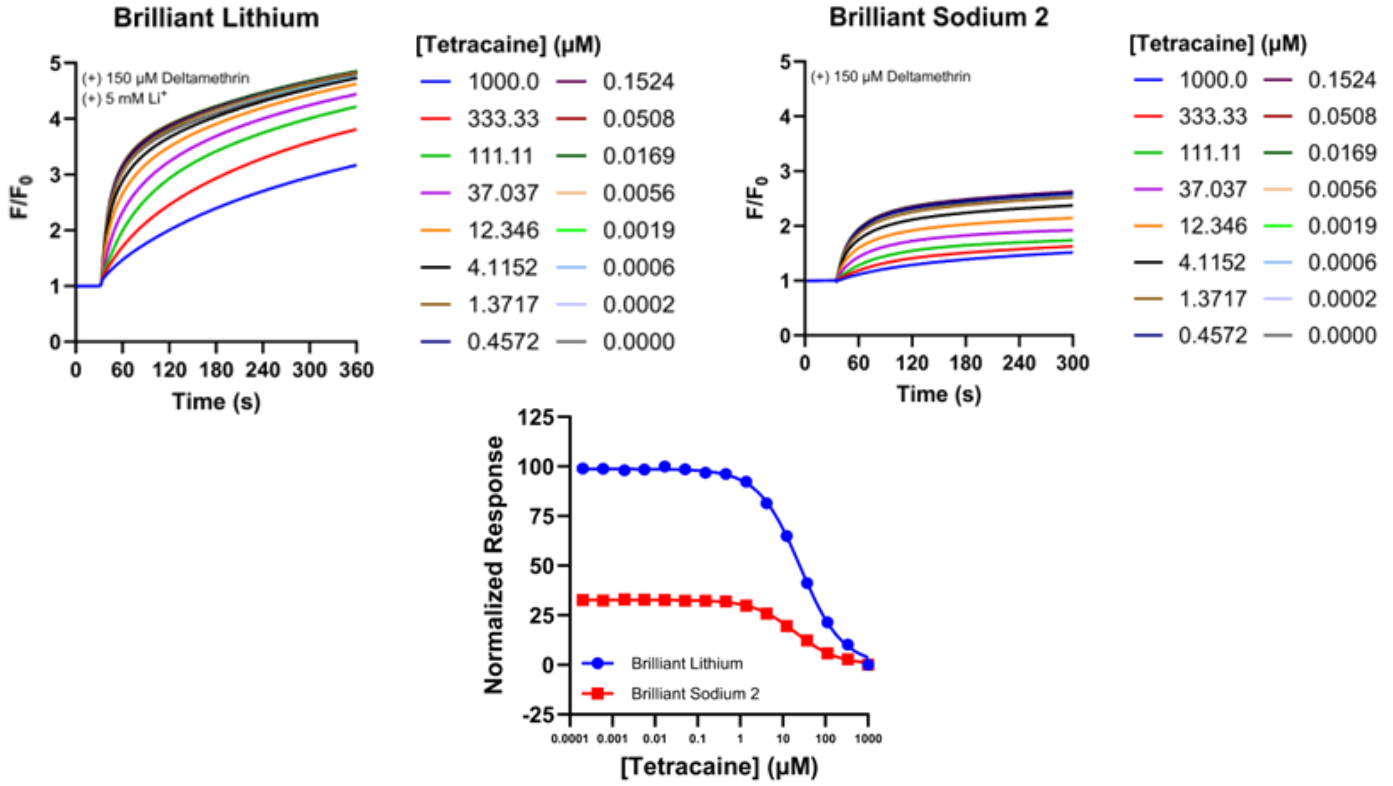

Figure 6. Comparisons of Brilliant Lithium and Brilliant Sodium 2 and on $Na_V1.8/\beta1$-expressing HEK293T Cells. Baseline-normalized kinetic data was collected on Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). ION Biosciences' Brilliant Lithium and Brilliant Sodium kits were conducted according to the products protocols. Tetracaine, a general pore blocker of $Na_V1.X$ channels, was added at 30 seconds alongside 150 µM deltamethrin. Brilliant Lithium produced over 2X the signal window of Brilliant Sodium 2.

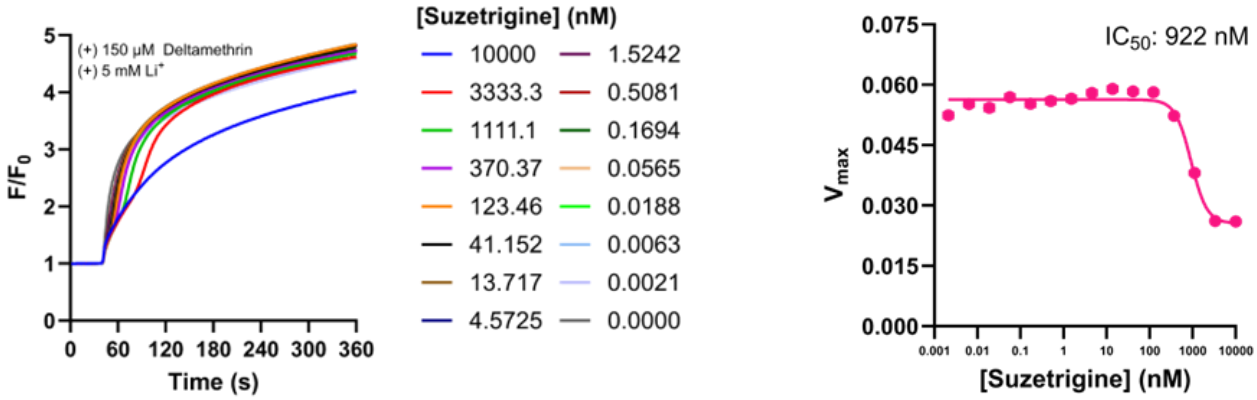

Figure 7. Suzetrigine Inhibition of $Na_V1.8/\beta1$-expressing HEK293T Cells. Baseline-normalized kinetic data was collected on Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). Suzetrigine, a potent inhibitor of $Na_V1.8$, was pretreated for 15 minutes. Deltamethrin, a potent activator of $Na_V1.8$ channels, was added at 30 seconds with 5 mM Li^+. The V_{max} was calculated from the initial slopes following stimulus addition. Error bars indicate the SEM (n = 3). The measured IC_{50} for suzetrigine inhibition of $Na_V1.8/\beta1$ is 922 nM.

Additional Information

Additional 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 7	Additional Reagents	Available Sizes	
Kit Label	**Name**	**Size**	**Catalog #**
Reagent A	ILG-1 AM	500 µg x 1 Vial 50 µg x 10 Vials 50 µg x 3 Vials	2215C 2215F 2215G
Reagent C	50X DySolv	20 mL Bottle	7501A
Reagent D	10X Brilliant Lithium Assay Buffer	Not a Regular Catalog Item. Contact Sales About Additional Purchase.	
Reagent E	50X TRS	20 mL Bottle	7060A
Reagent F	50X Probenecid	20 mL Bottle	7300P-50
Reagent G	10X Sodium Stimulus Buffer $[Na^+]$ = 1.38 M, $[Li^+]$ = 0 M	Not a Regular Catalog Item. Contact Sales About Additional Purchase.	
Reagent H	10X Lithium Stimulus Buffer $[Na^+]$ = 0 M, $[Li^+]$ = 1.38 M	Not a Regular Catalog Item. Contact Sales About Additional Purchase.	

ION Assurance Policy

We guarantee product quality. If you're unsatisfied with your order, send us an email at support@ionbiosciences.com. We'll promptly address your concern and replace, credit, or refund items according to our ION Assurance terms and conditions.