Brilliant Sodium 2 Assay

Catalog No. 9100

Introduction

Voltage-gated sodium channels ($Na_V1.X$) are essential proteins that enable the rapid influx of sodium ions and initiate action potentials in response to membrane depolarization. These electrical signals drive critical physiological processes, including neuronal communication ($Na_V1.1$, 1.2, and 1.6), cardiac conduction ($Na_V1.5$), and pain perception ($Na_V1.7$–1.9). Dysregulated $Na_V1.X$ activity is linked to disorders such as epilepsy, arrhythmias, and chronic pain, making these channels valuable therapeutic targets.

Traditional methods for assessing $Na_V1.X$ channel function, such as patch-clamp electrophysiology and radiolabeled ion flux assays, are often low-throughput, technically demanding, or require specialized equipment. ION Biosciences' Brilliant Sodium 2 Assay is a second-generation, fluorescence-based solution designed for high-throughput screening of $Na_V1.X$ modulators. At its core is ION Natrium Green 2 AM (ING-2 AM), a membrane-permeable, sodium-sensitive, fluorescent dye that enables real-time, direct measurement of intracellular sodium channel activity.

Activation of $Na_V1.X$ channels by membrane depolarization or direct stimulation causes a rapid influx of sodium ions, leading to a robust fluorescence increase. This change provides a sensitive and quantitative readout of $Na_V1.X$ channel activity and pharmacological modulation. Compared to the original version, Brilliant Sodium 2 delivers larger signal amplitudes and an expanded screening window, making it well-suited for determining EC_{50} or IC_{50} values of $Na_V1.X$ activators and inhibitors. The assay is fully compatible with 96- and 384-well plate formats and supports scalable, high-throughput screening across diverse cell types and applications.

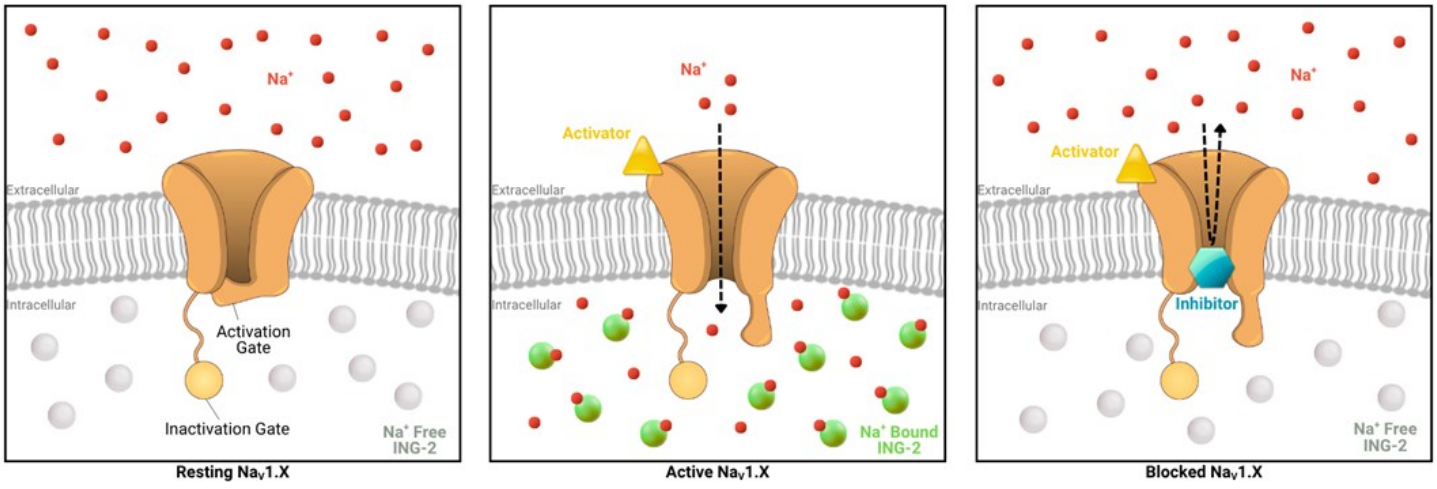

Figure 1. Brilliant Sodium 2 Assay Principles. Cells expressing voltage-gated sodium channels ($Na_V1.X$) are incubated with a Dye Loading Solution containing ING-2, a fluorescent indicator sensitive to intracellular sodium. Activation of $Na_V1.X$ channels by direct stimulation using veratridine ("activator") induces rapid sodium influx, leading to an increase in intracellular sodium. This change is detected as an increase in fluorescence, enabling a direct, real-time measurement of $Na_V1.X$ channel activity. When the channel is pre-treated with an inhibitor, veratridine-induced sodium influx is attenuated.

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# 9100-10		*pIONeer* Kit Cat# 9100-2		
Label	Name	Size	Qty	Size	Qty	Storage
Reagent A	ING-2 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 Sodium 2 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 Brilliant Sodium 2 Stimulus Buffer	10 mL Bottle	1	2 mL Bottle	1	4° C

Getting Started

Before beginning your experiment, ensure you have all the necessary reagents and materials. ION Biosciences' Brilliant Sodium 2 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. ING-2 AM should be protected from direct light and solutions containing ING-2 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 ING-2 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 Sodium 2 Assay Buffer (**Reagent D**) to the tube from **step 3**.
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**.

Procedure Continues on Next Page

Laboratory Procedures (Continued)

7. If desired add 200 µL of 50X Probenecid[2] (**Reagent F**) to the tube from **step 6**.
8. Add 20 µL of ING-2 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	ING-2 AM solution in DMSO	20 µL	20 µL
Reagent C	50X DySolv	200 µL	200 µL
Reagent D	10X Brilliant Sodium 2 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 Sodium 2 Assay Buffer (**Reagent D**) as shown in **Table 3** below. Briefly vortex to mix well.
11. 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.
12. Completely remove the cell-culture medium from the 96-well or 384-well microplate containing the cells of interest.

Table 3	Wash Solution[3] (Optional)	
Label	**Name**	**Volume**
Reagent D	10X Brilliant Sodium 2 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 Sodium 2 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 Sodium 2 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 2 Stimulus Buffer (**Reagent G**), and 4X concentrated $Na_V1.X$ activators as shown in **Table 5** below. Briefly vortex the **Stimulus Solution**[4] to mix well.
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**	**Volume**
Reagent G	10X Brilliant Sodium 2 Stimulus Buffer	1 mL
(Customer Supplied Material)	$Na_V1.X$ Activators	Enough for 4X concentration at total volume
	Water	Q.S. to total volume
	Total	10 mL

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).

Procedure Continues on Next Page

Laboratory Procedures (Continued)

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 ING-2 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**).
4. Using GraphPad® Prism, run the non-linear regression "agonist vs response" on the concentration-response curve (V_{max} or AUC) 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**).

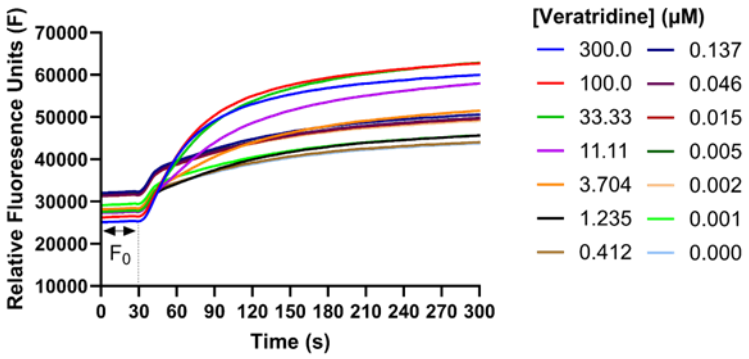

Figure 2. Raw Kinetic Data. Raw kinetic data of $Na_V1.3$-expressing HEK293 cells were collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). Relative Fluorescence Units (F) were collected for 30 seconds before the stimulus addition of various concentrations of veratridine. After addition, the plate continued to be read for an additional 270 seconds.

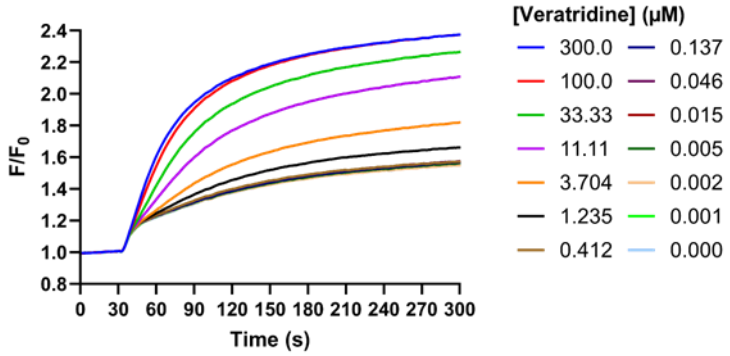

Figure 3. Baseline-Normalized Kinetic Data. Based on the raw kinetic data from **Figure 2**, average the relative fluorescence units (F) of the initial 30 seconds before stimulus addition and define it as the baseline (F_0). Calculate the ratio F/F_0 for each data set and plot F/F_0 over time as the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

Data Analysis (Continued) & Example Results

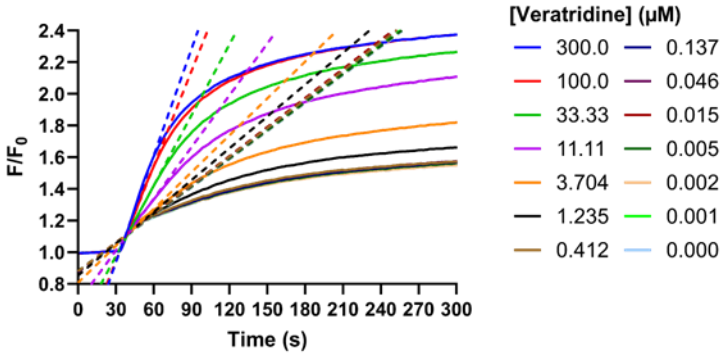

Figure 4. Maximum Velocity (V_{max}) Calculation. Based on the Baseline-Normalized Kinetic Data from **Figure 3**, conduct a linear regression on the first 30 seconds after stimulus addition. Calculate the slope and define it as the V_{max}.

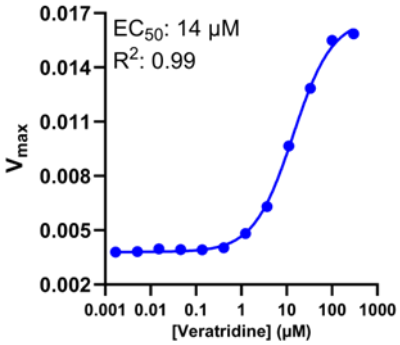

Figure 5. EC_{50} Calculation. Plot the V_{max} or the AUC against the compound concentrations and run the non-linear regression "agonist vs response" in GraphPad® Prism to calculate an EC_{50}. Alternatively, run the non-linear regression "inhibitor vs response" to calculate an IC_{50}.

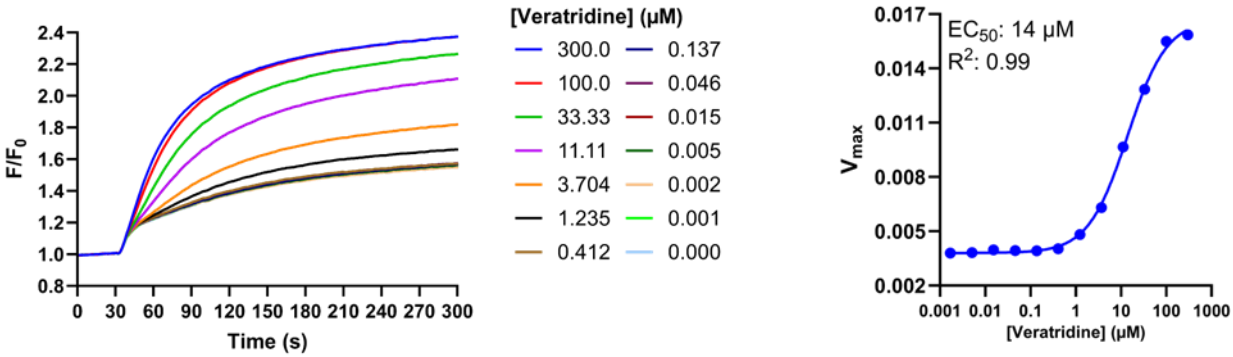

Figure 6. Veratridine Activation in $Na_V1.3$-Expressing HEK293 Cells. Baseline-normalized kinetic data was collected on Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). Veratridine, a potent activator of $Na_V1.X$ channels, was added at 30 seconds. The V_{max} was calculated from the first 30 seconds following stimulus addition. Error bars indicate the SEM (n = 3). The EC_{50} for veratridine activation of $Na_V1.3$ channels is about ~14 µM.

Example Results (Continued)

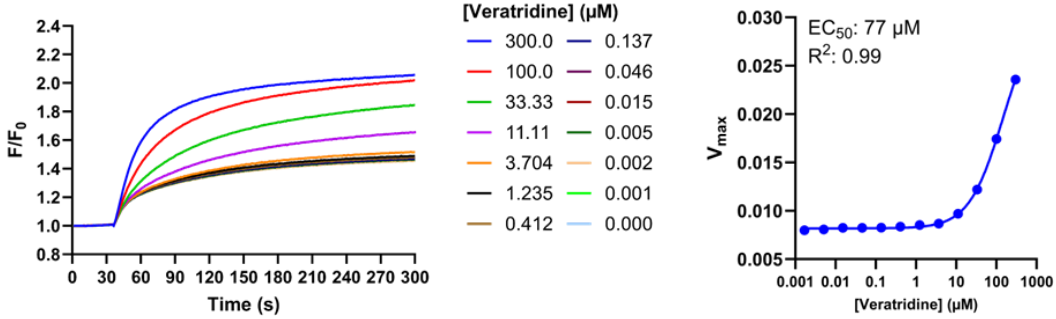

Figure 7. Veratridine Activation in $Na_V1.5$-Expressing CHO-K1 Cells. Baseline-normalized kinetic data was collected on Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). Veratridine, a potent activator of $Na_V1.X$ channels, was added at 30 seconds. The V_{max} was calculated from the first 30 seconds following stimulus addition. Error bars indicate the SEM (n = 3). The EC_{50} for veratridine activation of $Na_V1.5$ channels is about ~77 µM.

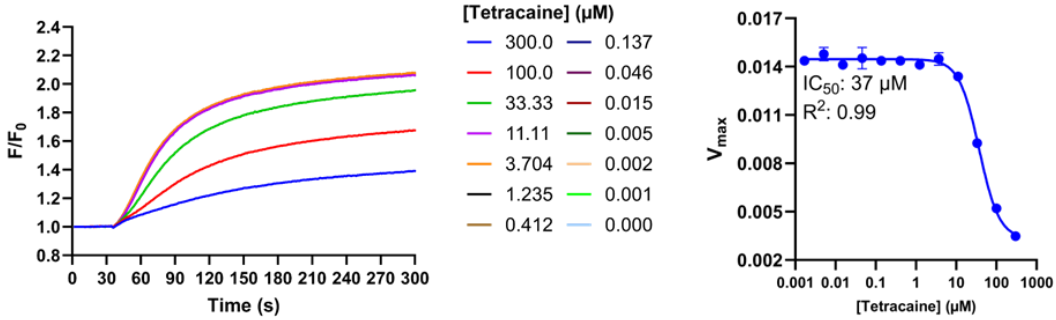

Figure 8. Tetracaine Inhibition of $Na_V1.3$-Expressing HEK293 Cells. Baseline-normalized kinetic data was collected on Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). Tetracaine, a potent inhibitor of $Na_V1.X$ channels, was added 15 minutes before the start of the assay. Veratridine, a potent activator of $Na_V1.X$ channels, was added at 30 seconds at a concentration of 100 µM. The V_{max} was calculated from the first 30 seconds following stimulus addition. Error bars indicate the SEM (n = 3). The IC_{50} for tetracaine inhibition of $Na_V1.3$ channels is about ~37 µM.

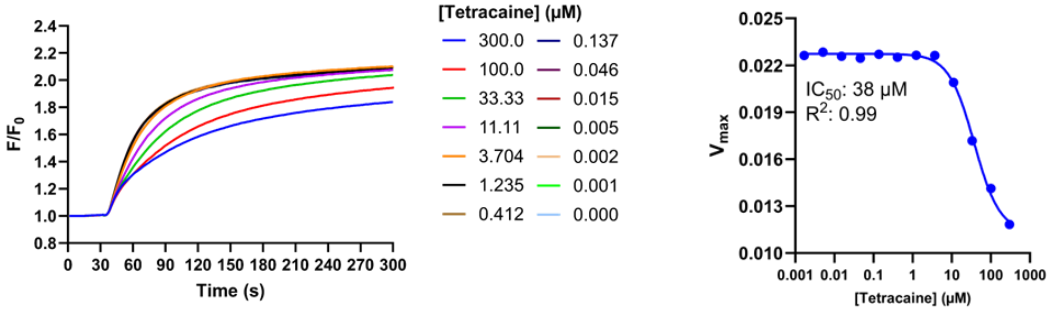

Figure 9. Tetracaine Inhibition of $Na_V1.5$-Expressing CHO-K1 Cells. Baseline-normalized kinetic data was collected on Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). Tetracaine, a potent inhibitor of $Na_V1.X$ channels, was added 15 minutes before the start of the assay. Veratridine, a potent activator of $Na_V1.X$ channels, was added at 30 seconds at a concentration of 300 µM. The V_{max} was calculated from the first 30 seconds following stimulus addition. Error bars indicate the SEM (n = 3). The IC_{50} for tetracaine inhibition of $Na_V1.5$ channels is about ~38 µM.

Example Results (Continued)

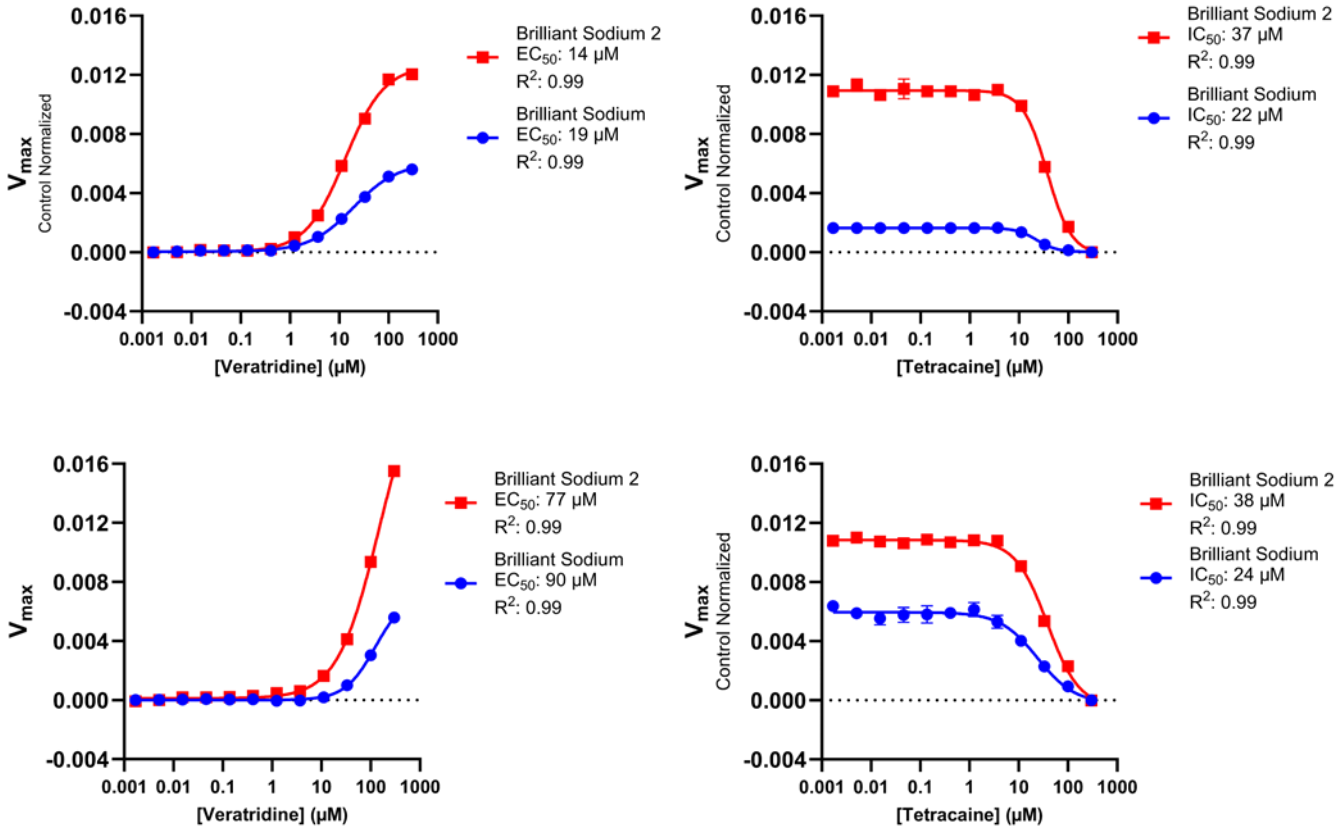

Figure 10. Kit Comparisons on $Na_V1.3$-expressing HEK293 cells and $Na_V1.5$-expressing CHO-K1 cells. Baseline-normalized kinetic data was collected on Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm) using ION Biosciences' Brilliant Sodium Assay and Brilliant Sodium 2 Assay. The signal window of the Brilliant Sodium 2 Assay is between 2-5X larger.

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	ING-2 AM	500 µg x 1 Vial	2011C
		50 µg x 10 Vials	2011F
		50 µg x 3 Vials	2011G
Reagent C	50X DySolv	20 mL Bottle	7501A
Reagent D	10X Brilliant Sodium 2 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 Brilliant Sodium 2 Stimulus Buffer	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.