

# Membrane Potential Assay to Identify Ion Channel Modulators

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## Abstract

Ion channels are challenging targets in the early phases of the drug discovery process, especially due to the lack of technologies available to screen large numbers of compounds in functionally relevant assays. Electrophysiological techniques, which are the gold standard for studying ion channels, are low throughput and not amenable to screening large numbers of compounds. Activation of ion channels leads to flux of ions through the channel across the cell membrane resulting in concomitant transient change in membrane potential. Both of these activities of ion channels are being exploited for the development of functional assays for ion channel screening. Development of fluorescent dyes sensitive to membrane potential made it possible to develop these assays for high throughput screening of ion channel targets. Two assay formats, FRET dye pair in Hamamatsu (FDSS6000) and blue membrane potential dye in FLIPR 384 for Nav1.7 channels are compared.

## Membrane Potential Assay: Sodium Channels

Activation of sodium channels leads to flux of Na ions through the channel across the cell membrane resulting in concomitant change in membrane potential which can be measured by using fluorescent dyes.

VSP FRET pair measures the change in membrane potential by monitoring the Fluorescence Resonance Energy Transfer (FRET) between the dye pair. The ratio of the donor emission to acceptor emission increases upon depolarization as measured in Hamamatsu (FDSS6000).

The feasibility of using MDC blue membrane potential dye and FLIPR to measure changes in membrane potential upon sodium channel activation was also explored.

## Methods

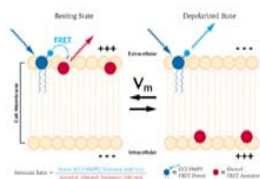
### Blue Membrane Potential Dye in FLIPR

- HEK-Nav1.7 cells were plated in poly-D lysine coated plates and grown to confluence.
- Cell were washed with assay buffer (1 mM MgSO<sub>4</sub>, 1 mM CaCl<sub>2</sub> in PBS). Blue Membrane potential dye (20 μL/well) was added and cells were incubated at 37°C, 45-60 min.
- At the end of incubation with dye, known sodium channel blockers were added to cell plate (10 μL/well).
- Agonist veratridine and Scorpion Venom (10 μL/well) is prepared in assay buffer and added on FLIPR384 (Dye is excited at 488 nm wavelength of the argon ion laser).

### VSP dye pair in Hamamatsu FDSS 6000

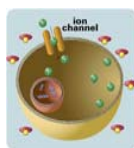
- HEK-Nav1.7 cells were plated in poly-D lysine coated plates and grown to confluence
- Cell were washed with assay buffer (Hank's-HEPES), 24 μL CC2DMPE dye (final conc. 15 μM) was added and cells were incubated at room temp for 30 minutes. Cells were then washed with assay buffer.
- 2.5 μM DiSBaC<sub>2</sub>(3) (final conc.) was added to cells and incubated for 20 min at 37°C.
- Sodium channel blockers (15 μL) were added to the cells 10 minutes prior to putting the plate in Hamamatsu (FDSS6000)
- Agonist veratridine and Scorpion Venom (25 μL/well) was added by the Hamamatsu pipettor (Response ratio is emission at 460/580)

### Mechanism of VSP dye pair



Courtesy of Invitrogen

### Membrane Potential Sensitive Dye



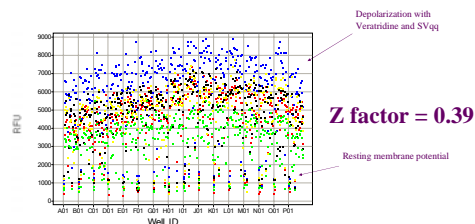
Courtesy of Molecular Devices

## Membrane Potential Assay: FRET dye pair (Hamamatsu FDSS6000)

		DISBaC <sub>2</sub> (3)							
		20	10	5	2.5				
CC2-DMPE	20	0.05	1.14	0.07	0.82	0.03	0.52	0.04	0.30
	10	0.05	1.37	0.07	1.13	0.02	1.01	0.02	0.53
	5	0.05	1.61	0.06	1.38	0.04	1.12	0.06	0.66
	2.5	0.07	1.70	0.02	1.60	0.08	1.42	0.09	0.73

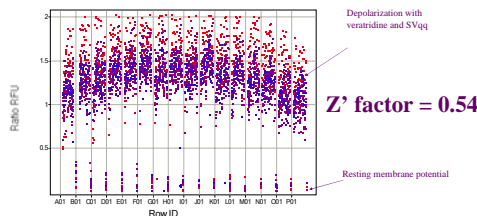
Matrix of VSP-1 Dyes Using Recombinant Cells expressing Na Channel. Each dye concentration in μM is indicated. Response following agonist addition is bold, buffer addition only is left in plain text, expressed as the maximum change in the 465-580 ratio. Red bar indicates the dye concentrations used for subsequent experiments.

## Membrane Potential Assays: Blue Membrane potential dye (FLIPR 384)



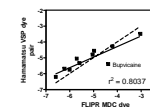
- After loading the dye HEK-Nav1.7 cells were treated with 25 μM Veratridine and 1 μg/ml Scorpion Venom OQ.
- Each plate is represented in different color.

## Sodium Channels: FRET dye pair (Hamamatsu FDSS6000)



- After loading the dye HEK-Nav1.7 cells were treated with 25 μM Veratridine and 1 μg/ml Scorpion Venom OQ.
- Each plate is represented in different color.
- The edge effect seen here was due to loss of cells during cell wash.

## Sodium Channel Blockers: Membrane Potential Assays (Nav1.7 Channel)

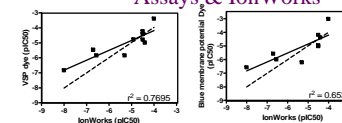


The solid line is the line of correlation and the dotted line is the line of one-to-one correspondence

IC<sub>50</sub> of known sodium channel blockers were similar in both membrane potential assays using VSP dye pair and MP dye

Blocker	FLIPR	FRET (Hamamatsu)	IC <sub>50</sub>
	IC <sub>50</sub> (μM)	IC <sub>50</sub> (μM)	
Lidocaine	3.83	3.59	4.00
Rupatadine	4.38	5.49	4.49
Quinidine	4.39	4.29	4.59
Procainamide	4.37	4.57	4.50
TTX	6.58	6.21	6.00
Flunarizine	5.58	5.26	5.70
Mexiletine	4.97	4.57	4.59
Phenytoin	5.63	4.21	4.50
Propafenone	6.21	5.70	5.30
Tetracaine	6.00	5.79	6.55
CC-10000	5.79	6.06	

## Correlation: Membrane Potential Assays & IonWorks



The solid line is the line of correlation and the dotted line is the line of one-to-one correspondence

In general sodium channel blockers with higher potency in electrophysiology (IonWorks) appear less potent in both membrane potential assays.

## Membrane Potential Assay: Nav1.7 channel

### Conclusions:

In HEK-Nav1.7 cell line Nav1.7 channels were activated by treating the cells with 25 μM Veratridine and 1 μg/ml SVqq. This response was of suitable magnitude for quantitative measures in a 384-well microtiter plate format.

The optimal assay conditions yielded a S:B of 8-10:1 and a Z' ≥ 0.5 using FRET dye pair and S:B ~4:1; Z' ~0.4 using blue membrane potential dye.

The specificity of this response was demonstrated using Na<sup>+</sup> channel blockers (albeit nonselective) and showed a lack of effect in non-transfected cells.

These results indicate that pharmacological modulation of Nav1.7 channels could be measured using membrane potential dye (FRET pair) in Hamamatsu FDSS6000.

Although the IC<sub>50</sub> of potent sodium channel blockers is lower in membrane potential assay relative to values obtained in electrophysiology assay, FRET dye pair membrane potential assay is robust enough to identify novel sodium channel blockers in high throughput screening