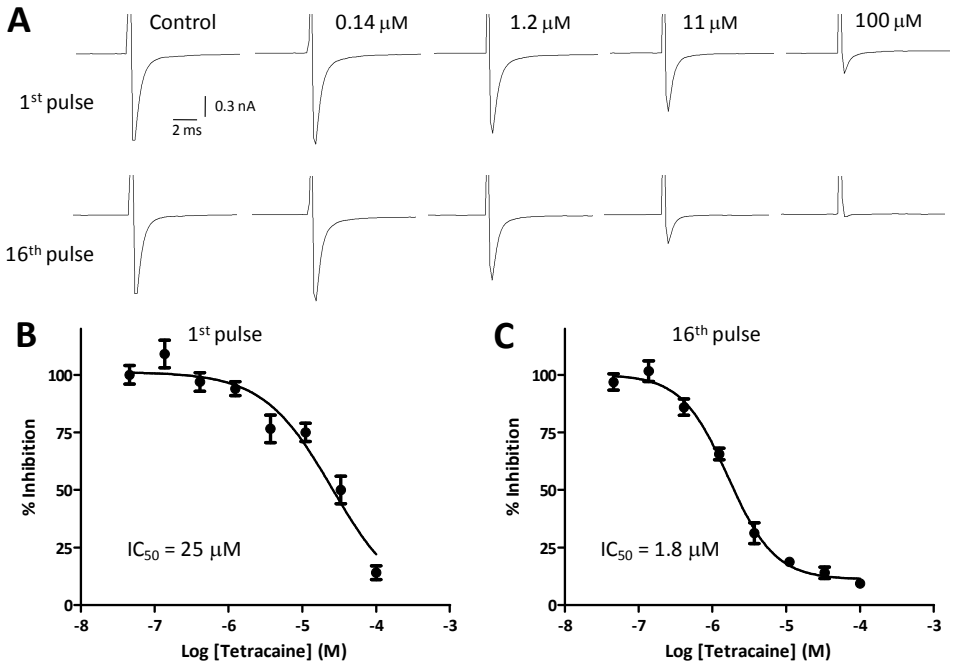
Assay Snapshot

hNa$_V$1.5 automated patch-clamp electrophysiology assay

- **Assay** hNa$_V$1.5 automated patch clamp electrophysiology
- **Channel** Cardiac voltage-gated Na^+ channel, Na$_V$1.5
- **Gene Name** *SCN5A* (Ref seq. NM_000335)
- **Synonyms** hH1, SKMII, LQT3
- **Assay format** 384-well IonWorks Quattro Population Patch Clamp electrophysiology
- **Cell Host** Human Embryonic Kidney (HEK-293)
- **Stimulus** Repeated gating steps, V_h -90 mV, V_{step} +15 mV, 1 or 3 Hz
- **Controls** Tetracaine, 0.3% DMSO



Pharmacology Use-dependent inhibition of hNa$_V$1.5 cardiac Na^+ currents by tetracaine. Currents were evoked by trains of depolarizing pulses (+15 mV, 50 ms duration, 3 Hz, 16 pulses) from a V_h of -90 mV.

Background

Cardiac Na^+ channels ($Na_V1.5$) underlie regenerative potentials that initiate contractions in cardiac myocytes and mediate electrical transmission in cardiac fibers that relay electrical signals across the heart. Genetic mutations that reduce function of $Na_V1.5$, as well as small molecule blockers that interact with $Na_V1.5$, can disrupt electrical conduction and contraction of the heart, leading to serious adverse cardiac events, including ventricular fibrillation and sudden cardiac death. While targeted as anti-arrhythmics, off-target interactions with $Na_V1.5$ channels can be problematic and result in potential cardiac liabilities.

Pharmacology Summary

IC_{50} values for inhibition of $hNa_V1.5$ currents (1st and 16th pulse in a 3 Hz train)

Compound	IC_{50} – 1st Pulse (µM) *	IC_{50} – 16th Pulse (µM) *
Amitriptyline	8 ± 1	1 ± 0.4
Tetracaine	22 ± 5	2 ± 0.6
Propafenone	9 ± 3	4 ± 1
Propranolol	35 ± 5	6 ± 3
Bupivacaine	84 ± 26	9 ± 4
Verapamil	84 (n=1)	9 ± 3
Flecainide	24 ± 13	13 ± 7
Quinidine	33 ± 10	14 ± 6
Mexiletine	91 ± 43	40 ± 9
Lidocaine	437 ± 291	102 ± 67
Procainamide	4800 ± 1200	2000 ± 700
Tocainide		23 ± 11% inhibit @ 100 µM
Dofetilide		No inhibit @ 10 µM
Sotalol		No inhibit @ 3 mM

Assay QC

Cell, Curve fit, Solubility & Plate QC parameters applied:

Cell: $I_{hNaV1.5}$ >400pA, baseline & seal resistance stability filters
Plate QC: Minimum 345 cells, Tetracaine pIC_{50} 4.4 - 5.0 (1st pulse) and 5.4 – 6.0 (16th pulse)
Typical assay precision: compound pIC_{50} values ± 0.25 log units

Why Essen?

The voltage-clamp protocols for the $Na_V1.5$ assay were designed with the endpoint in mind: sensitivity and translation. Using the IonWorks $Na_V1.5$ assay, data with a set of reference small molecule Na^+ channel blockers show sensitivity and rank order comparable with conventional voltage-clamp electrophysiology and in alignment with free therapeutic plasma levels associated

with efficacy or side-effects on cardiac Na_V channels (data upon request). As with all of our assays we offer high fidelity data at competitive pricing with rapid data turnaround times to facilitate project decisions.

References

Makielski, J.C. et al., (2003). A ubiquitous splice variant and a common polymorphism affect heterologous expression of recombinant human SCN5A heart sodium channels. *Circ. Res*. **93**, 821-828.

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