THE ION CHANNEL EXPERT

Stably Transfected Cell Line Data Sheet
$hCa_v3.2$-HEK
Catalog Number CT6106

Related Services and Products

FastPatch® and ScreenPatch™ automated patch clamp services
Additional information available at www.chantest.com

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1 Cell Line Description

1.1 Background

Cav3.2 is a voltage-gated, Ca^{2+}-selective ion channel responsible for T-type calcium current in cardiac and smooth muscle, neuroendocrine and neuronal cells. Cav3.2 channels regulate repetitive pacemaking and repetitive firing, and are therapeutic targets in cardiovascular and neurological disorders.

1.2 Pore-forming subunit identifier: $hCa_v3.2$

Class: Voltage-gated calcium channel
Species: Human
Synonyms: T-type calcium channel
Gene name: CACNA1H

1.3 Sequence Information

The cDNA sequence of the CACNA1H gene used to create this cell line was confirmed prior to transfection. The amino acid sequence encoded by the transfected cDNA is identical to the translated sequence for GenBank accession number NM_021098.2.

1.4 Expression System

HEK293 (human embryonic kidney cells), tetracycline-inducible expression.

1.5 Product Format

Cryopreserved cells, 1 $x10^6$ cells/vial

1.6 Mycoplasma Status: Negative

The absence of mycoplasma species in this cell line was confirmed with the MycoAlert Kit (Lonza Rockland, Inc.).

1.7 Cell line Stability

Channel expression in this cell line has been shown to be stable in patch clamp assays for at least 29 passages.

2 Validated Test Platforms

Electrophysiological and pharmacological verification of the functional properties of the cloned channels was assessed in the following test platforms:

Manual Patch Clamp
PatchXpress® (MDS-AT)
QPatch™ HT (Sophion)
FLIPR® (MDS-AT)

2.1 Representative Manual Patch Clamp Data

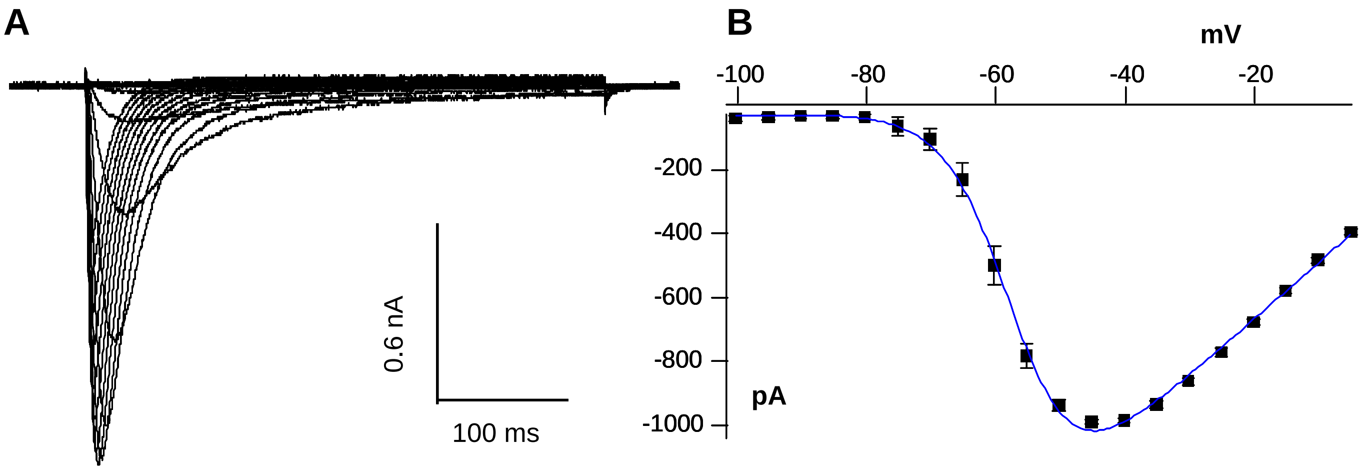

Figure 1. Voltage-Dependent Activation in Manual Patch Clamp
A: Family of current traces elicited by test pulses -100 to 0 mV, in 5 mV increments, holding potential -80 mV. **B:** Peak I-V relationship. Mean ± SEM, n = 10 cells.

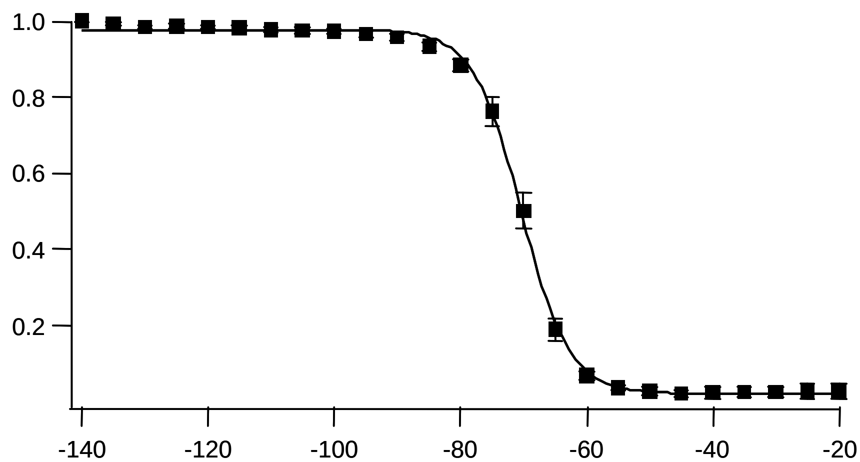

Figure 2. Voltage-Dependence of Inactivation
Currents were elicited by a 500- ms variable amplitude conditioning pulse (-140 to -20 mV, in 5 mV increments) was followed by a 30-ms fixed amplitude (-30 mV) test potential. Test pulse current amplitude (normalized to maximum current, Mean ± SEM, n = 8 cells) is plotted versus conditioning pulse amplitude to assess the fraction of non-inactivated channels. The midpoint potential was -70.3 ± 0.6 mV.

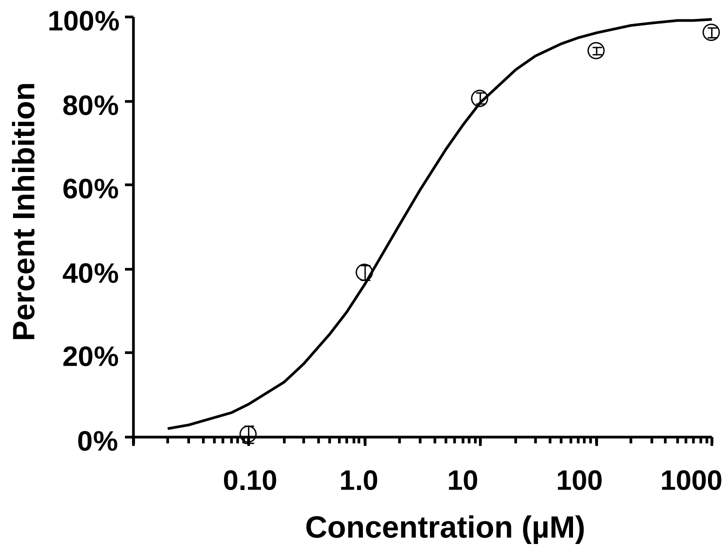

Figure 3. Ni^{2+} Block of $hCa_v3.2$ in Manual Patch Clamp
Concentration–response relationship in hCav3.2-HEK cells (Mean ± SEM, n = 3 - 6 cells/concentration). IC_{50} = 2 μM.

2.2 PatchXpress®

2.2.1 Throughput Capability in PatchXpress®

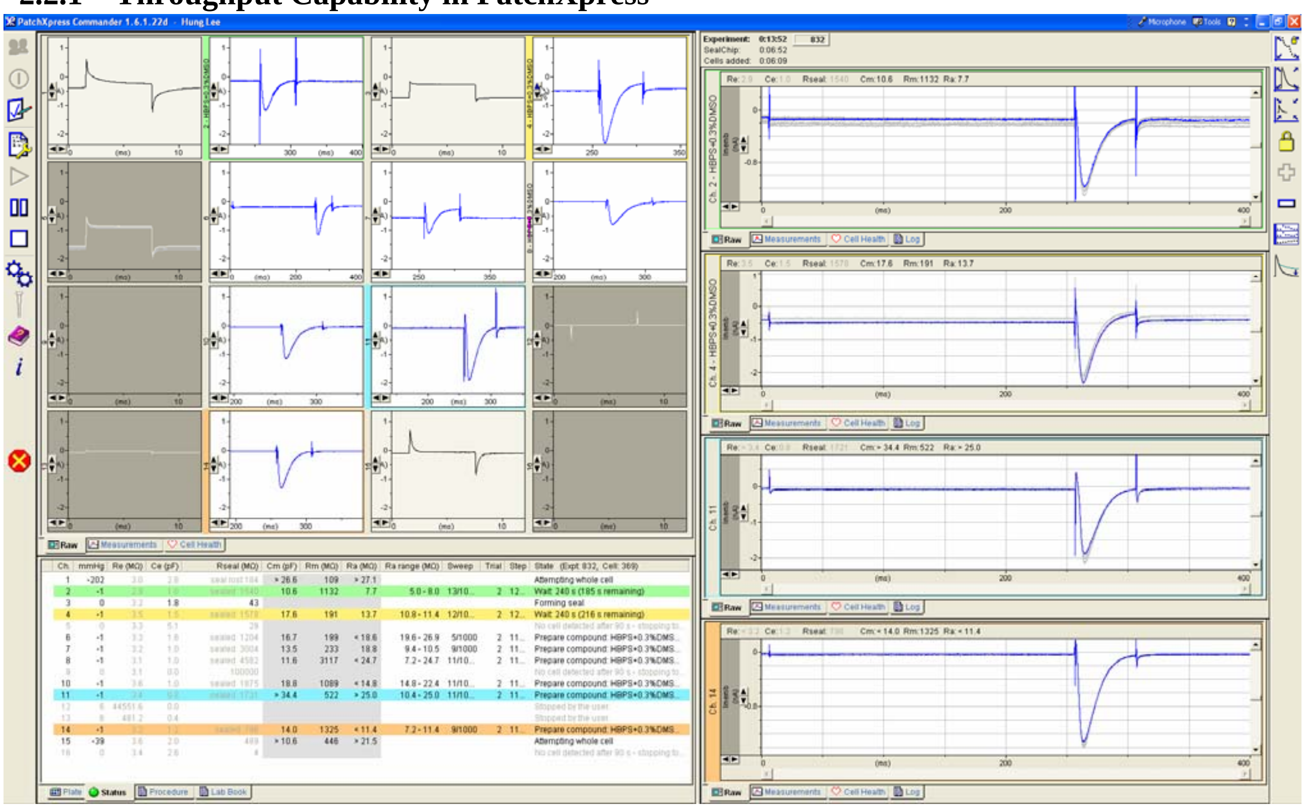

Figure 4. PatchXpress® Throughput Capability

Throughput in PatchXpress® depends upon many factors that introduce variability in success rates. The screen capture shows a typical PatchXpress® experiment. In this example, 8 of a possible 16 seals were formed; whole-cell configuration was achieved in 8 cells with expected $Ca_v3.2$ current waveforms.

2.2.2 Representative Data

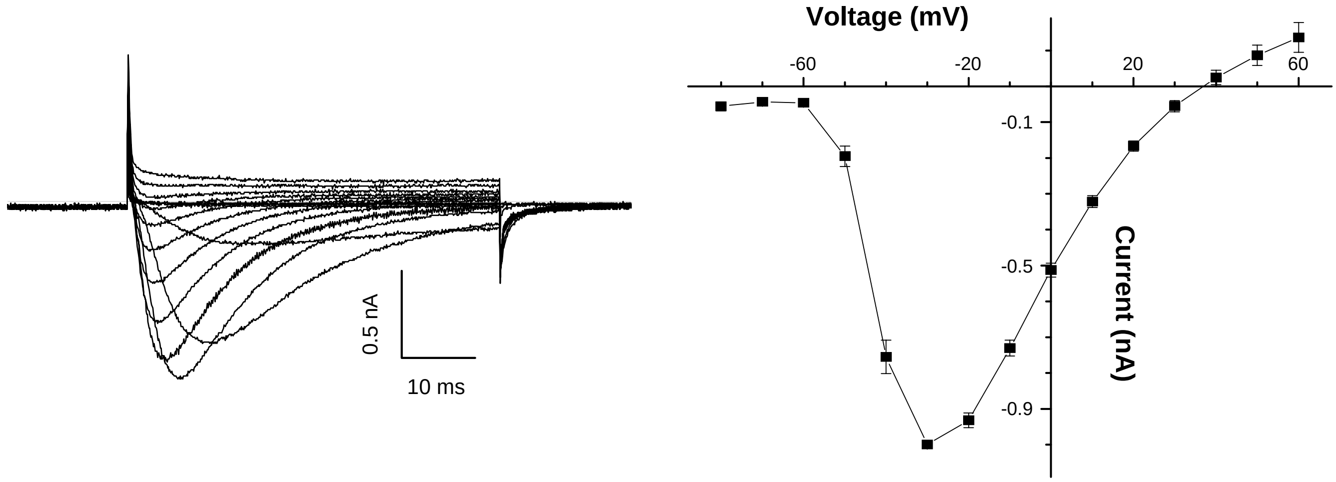

Figure 5. Voltage-Dependent Gating Characteristics in PatchXpress®
A: Family of current traces elicited by test pulses from -80 to +60 mV in 10 mV increments, holding potential -80 mV. **B:** Peak current-voltage relationship (Mean ± SEM, n = 4 cells).

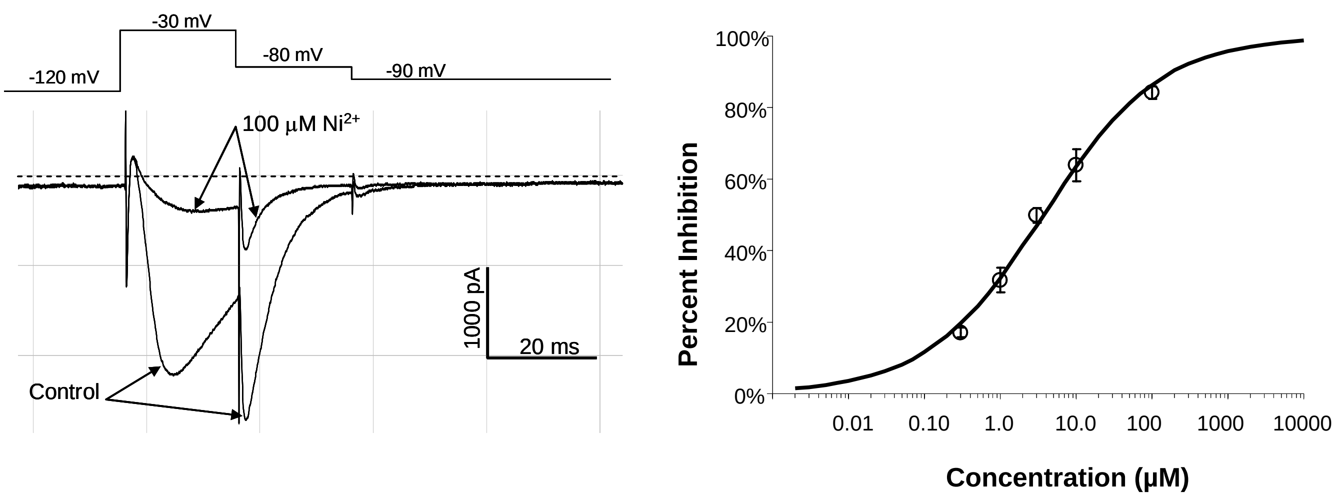

Figure 6. Ni^{2+} block of $hCa_v3.2$ current in PatchXpress®
A: Test pulse currents at -30 mV before and after 100 µM Ni^{2+} application.
B: Concentration-response relationship (Mean ± SEM, n = 4 - 6), IC_{50} = 3.7 µM.

2.3 Representative QPatch™ HT Data

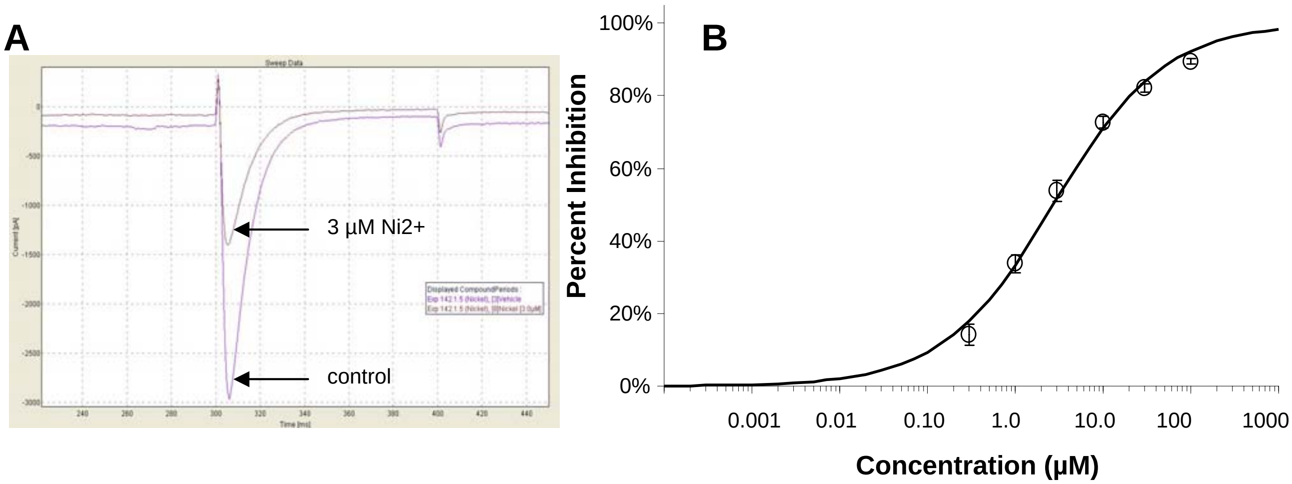

Figure 7. Ni^{2+} block of hCa$_v$3.2 current in QPatch™ HT
A: Test pulse currents before (control) and after 3 µM Ni^{2+} application.
B: Concentration-response relationship (Mean ± SEM, n = 9 - 14 cells/concentration). IC_{50} = 2.7 µM.

2.4 Representative FLIPR Tetra® Data

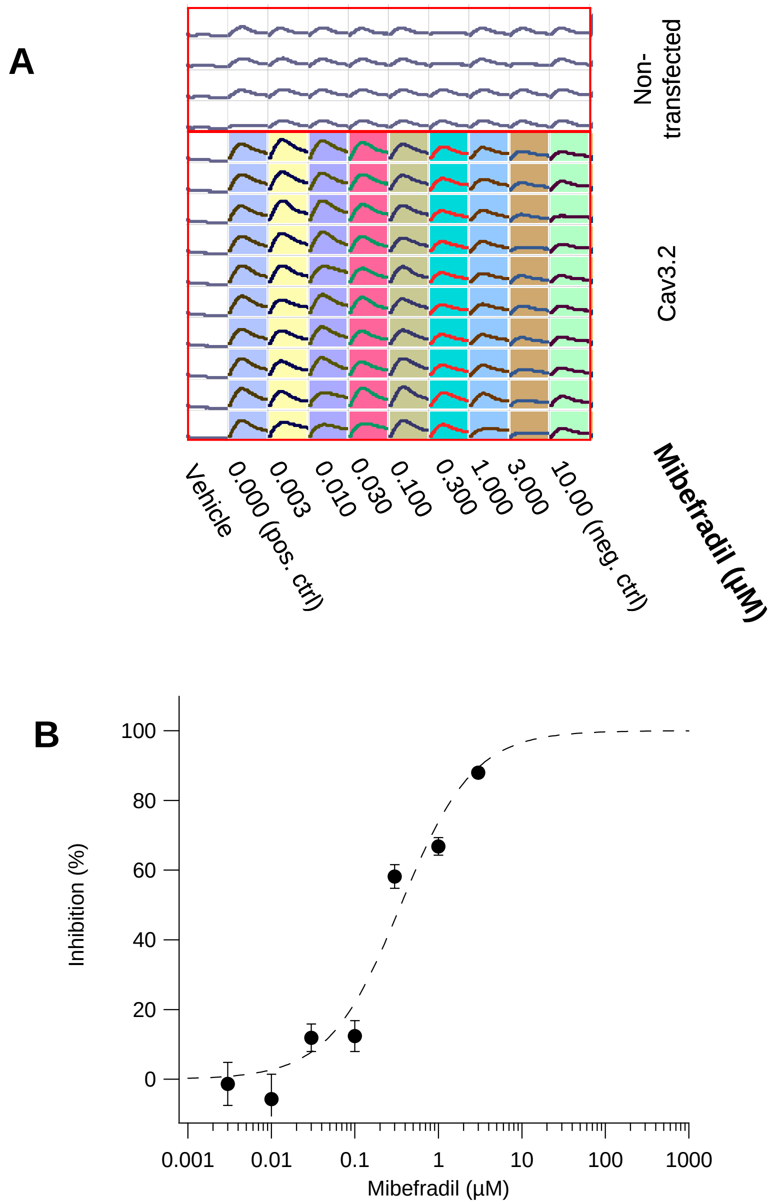

Figure 8. Mibefradil Inhibition of hCa$_v$3.2 in FLIPR®
A: Fluorescent $[Ca^{2+}]_i$ signals evoked by K^+-depolarization were inhibited by mibefradil in a concentration-dependent manner. **B:** Concentration-response relationship (Mean ± SEM, n = 7 - 12 replicates/concentration). IC_{50} = 0.4 µM.

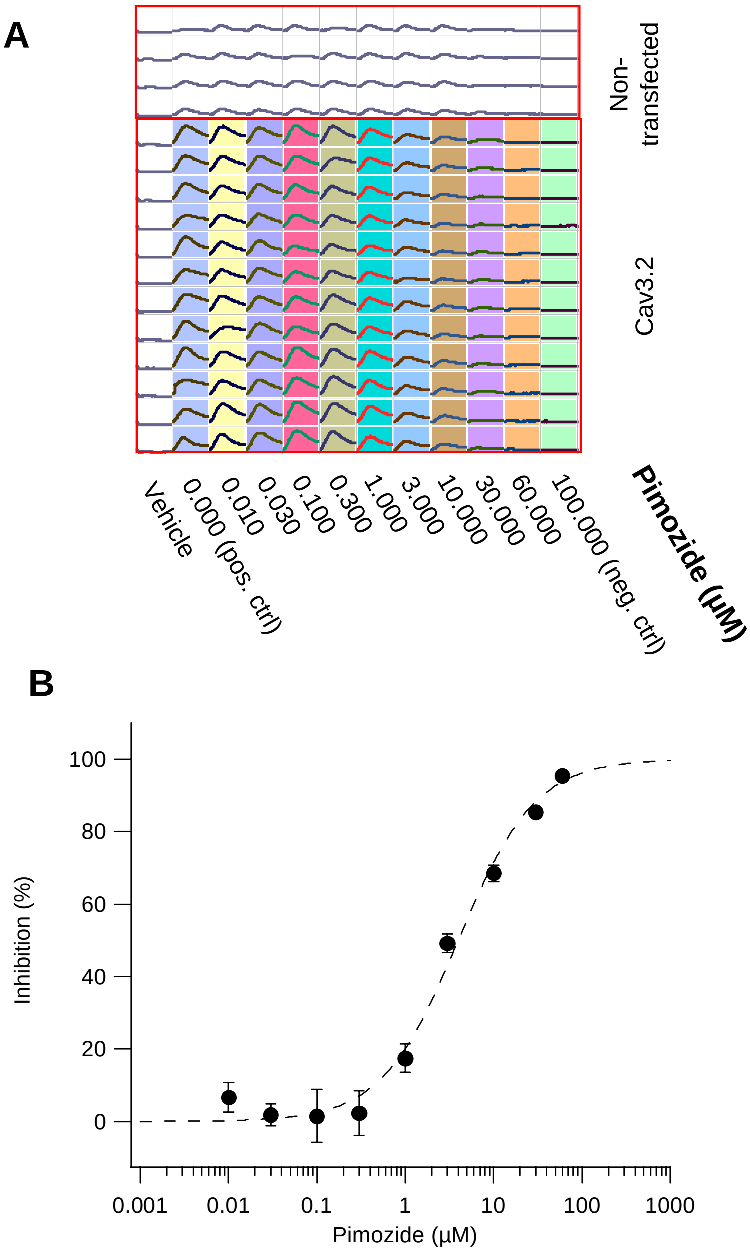

Figure 9. Pimozide inhibition of Ca$_v$3.2
A: Intracellular calcium signals evoked by Ca$_v$3.2 activation were inhibited by increasing concentrations of pimozide. **B:** Concentration-response relationship (Mean ± SEM, n = 7 - 12 replicates/concentration). IC_{50} = 4 µM.

3 References

Catterall WA et al. 2005. Pharmacol Rev. 57:411-25. International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels.

Perez-Reyes E. 2003. Molecular physiology of low-voltage-activated T-type calcium channels. Physiol Rev 83:117-161.