PRODUCT DATASHEET

PrecisION™ hNav1.8/β1-HEK Recombinant Cell Line

Catalog Number: CYL3025

PRODUCT DESCRIPTION
Recombinant HEK293 cell line expressing the human Nav1.8 (accession number AF117907; tetrodotoxin-resistant voltage-gated sodium channel type X (SCN10A)) and the human sodium channel beta 1 subunit (accession number NM_001037; SCN1B).

ASSOCIATED PRODUCTS
The PrecisION™ hNav1.8/β1-HEK Recombinant Cell Line is provided to customers on the purchase of an appropriate license. The available licenses are:

- CYL3025SS  PrecisION™ hNav1.8/β1-HEK Single Site License
- CYL3025TS  PrecisION™ hNav1.8/β1-HEK Two Site License
- CYL3025MS  PrecisION™ hNav1.8/β1-HEK Multiple Site License

CONTENTS
2 x 1 mL aliquots containing 1.2 x 10^6 cells/mL in 10% DMSO at passage 17.

STORAGE
Vials are to be stored in liquid N₂.

WARNINGS
For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

GMO
This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismuja.
Denna produkt innehåller genetisk ändrade organismer.

MYCOPLASMA TESTING
The cell line has been screened using the ELISA based Mycoplasma Detection kit (Roche) and by a PCR VenorGem kit (Minerva Biolabs) to confirm the absence of Mycoplasma species.

FUNCTIONAL VALIDATION
The electrophysiological and pharmacological properties of the cell line were examined using conventional whole-cell recording techniques. The current voltage relationship and activation/inactivation kinetics were similar to previously reported data for hNav1.8/β1 currents (Vijayaragavan et al., 2001; 2003; Vijayaragavan et al., 2004)). The currents were resistant (insensitive) to block by 10 μM TTX. The current were also blocked by tetracaine, lidocaine and lamotrigine. Functional channel expression over time was monitored using PatchXpress™. At least 75% of cells expressed hNav1.8/β1 currents over 500 pA for 30 passages. The mean current was 900 ± 17 pA (n = 11).
RECOMMENDED CULTURE CONDITIONS
Recommended culture conditions and standard operating procedure are provided with the product.

INTRODUCTION
The voltage-gated sodium channel α-subunit Nav1.8 (also known as hSCN10A, SNS and PN3) is expressed in high levels in mammalian adult dorsal root ganglion (DRG) neurons where it is involved in action potential initiation and transmission of nociceptive impulses (Leffler et al., 2007). Native sodium channels are multi-subunit complexes, composed of not only the pore forming α subunit, but also auxiliary β subunits. The β1 subunit is known to increase peak current amplitude as well as increasing sodium ion channel expression at the cell surface (Vijayaragavan et al., 2004).

FUNCTIONAL VALIDATION
Electrophysiological Properties of the hNav1.8/β1 current.

Whole-cell Patch Clamp Electrophysiology.

Current/Voltage Relationship:

**Activation:** Activation of the hNav1.8/β1 produced an inward current with slow inactivation kinetics (Figure 1). The channel activated at approximately -40 mV and reached its peak current at 20 mV (Figure 2). The hNav1.8/β1 channel had a half-activation voltage ($V_{1/2}$) of -1.2 ± 0.7 mV and a slope ($k$) of 11.4 ± 0.6 (Figure 3). This is close to previously reported values ($V_{1/2} = -3$ to 2.5 mV and $k = 5.5 – 11.3$; Rabert et al., 1998; Vijayaragavan et al., 2001; 2003; Vijayaragavan et al., 2004).

**Figure 1.** Typical current traces for hNav1.8/β1 evoked by the depolarizing voltage steps. The currents were produced by 100 ms voltage steps from -90 to +60 mV in 10 mV increments. Sweep every 10 s. The cells were held at a holding potential of -90 mV. The voltage protocol used is shown in red.
Figure 2. Typical Current-voltage (I/V) relationship for hNav1.8/β1.

Figure 3. Conductance-voltage graph of the hNav1.8/β1 current. The conductance was normalized to peak conductance at +20 mV and plotted against membrane voltage. The voltage protocol used is shown (inset).
Inactivation:
The hNav1.8/β1 channel had a half-inactivation voltage ($V_i$) of $-47.6 \pm 0.9$ mV and a slope (k) of $9.5 \pm 0.8$ (Figure 4). Again this is close to values reported previously ($V_{1/2} = -40$ to $-63$ mV and $k = 7.1 - 12.9$; Vijayaragavan et al., 2001; Vijayaragavan et al., 2004; Leffler et al., 2007).

**Figure 4. Steady-state inactivation of the hNav1.8/β1.**
The voltage protocol used is shown (inset).
Recovery from inactivation:
In order to assess the time constant of recovery of inactivation at -110 mV following an inactivating 20 ms voltage step to 10 mV, a double pulse protocol was used. First, the membrane was stepped from the holding potential (-110 mV) to 10 mV for 20 ms (Pulse 1, P1). The holding potential of -110 mV was sufficient to remove any inactivation so that the subsequent test pulse to 10 mV produced maximal inward current (i.e. all channels were available for activation). Immediately after the step to 10 mV the voltage was returned to -110 mV for varying amounts of time ranging from 1 – 38.4 ms with each successive intervals increasing by 50% (interpulse duration). After each incremental change in time, the voltage was stepped back to 10 mV (Pulse 2, P2). This was to assess the amount of recovery from inactivation at each incremental time point at -110 mV, by comparing the amplitude of P2 (after returning to -110 mV) with P1. Clearly, if channels had fully recovered at a given incremental time point, then the amplitude of P2 would be the same as P1, hence P2/P1 = 1. However, if channels had only partially recovered at a given incremental time point, the amplitude of P2 would be smaller, so that P2/P1 = <1. This relationship is quantified in the graph for three cells, where P2/P1 is plotted against the relevant interpulse duration. The data could be described by a double exponential giving an estimated time constants of recovery where the predominant recovery time constant (approx 80% of total amplitude), $\tau_{fast} = 1.9 \pm 0.4$ ms and the slower time constant (20% of the amplitude), $\tau_{slow} = 8.2 \pm 13.2$ ms (Figure 5).

Figure 5. Recovery from inactivation of the hNav1.8/β1.
Pharmacological Properties of the hNav1.8/β1 current.

Pharmacology - Tetrodotoxin:
The puffer fish derived toxin, tetrodotoxin, has been found to block multiple sodium channels in the nanomolar range. The hNav1.8 channel is very resistant to block by TTX, with an IC\textsubscript{50} of 60 - 80 µM (Akopian \textit{et al.}, 1996; Rabert \textit{et al.}, 1998). In order to conclusively demonstrate the functional expression of hNav1.8 currents, 10 µM TTX was tested on the inward currents evoked by stepping to a test potential of 20 mV from a holding potential of -90 mV (Figure 6). Currents following the addition of 10 µM TTX were 93.4 ± 1.8\% (n = 11) of those preceding the addition of TTX.

Figure 6. The effect of 10 µM tetrodotoxin on hNav1.8/β1 currents.
Typical traces showing the effect of 10 µM tetrodotoxin. Sodium currents are shown before and in the presence of tetrodotoxin. Black trace represents inward current before addition of TTX and the red trace the current in the presence of TTX. The cells were held at a holding potential of -90 mV and then stepped to a test pulse to 20 mV for 20 ms at a frequency of 0.1 Hz.
Pharmacology – A-803467:
A-803467 is a sodium channel blocker that potently blocks hNav1.8 with an IC$_{50}$ in the nanomolar range whilst blocking other Nav channels in the micromolar range (Jarvis et al., 2007). hNav1.8 currents evoked by repetitive (10 Hz) 20 ms depolarizing pulses from a holding current of either –90 mV or –60 mV were blocked by 100 nM by 42-43% at -90 mV and by 62-70% at -60 mV (Figure 7).

Figure 7. The block of hNav1.8/β1 by 100 nM A-803467
The upper panel shows peak inward current plotted for each consecutive pulse, before, in the presence of A803467 (red bar) and after wash off of drug using a $V_{\text{hold}}$ of –90 mV (left) or –60 mV (right). The lower panel shows representative individual current traces at the indicated time points.
Pharmacology – Tetracaine, Lidocaine and Lamotrigine:
The potency of the block of the hNav1.8/β1 by tetracaine, lidocaine and lamotrigine when 50% of the channels are in the inactive state cells was assessed. Cells were voltage clamped at a holding potential of -90 mV then stepped to -50 mV (∼V½ inactivation) for 5 seconds followed by a test pulse to 20 mV. This was repeated every 10 seconds. hNav1.8/β1 was blocked with IC₅₀ values of 0.6 ± 0.2 µM (n = 9), 63.3 ± 12.2 µM (n = 6) and 202.3 ± 22.5 µM (n = 6) for tetracaine, lidocaine and lamotrigine respectively (Figure 8). These values are in agreement with published findings (Poyraz et al., 2003; Jarvis et al., 2007).

Figure 8. Block of hNav1.8/β1 current using tetracaine, lidocaine and lamotrigine.
A. Dose-response curves of tetracaine, lidocaine and lamotrigine on the hNav1.8/β1 current. Voltage protocol shown in inset.
B. Typical current traces showing the inhibition of hNav1.8 currents by increasing doses of lamotrigine. Control current before lamotrigine addition shown in black.
Stability of hNav1.8/β1–HEK293 Cell Line.

PatchXpress Electrophysiology.

The hNav1.8/β1–HEK293 cell line has stable expression for >30 passages.

Figure 8. Stability of expression over passage.
The upper panel shows the percentage of cells expressing a mean peak inward current >500 pA for cell passages 9, 14, 19, 26. The numbers above each bar represent the number of cells sealed (out of a maximum of 16).
Vector:

Polylinker: CMV-BamHI-NotI-hNav1.8-NotI-Ascl-NdeI-EcoRI-IRES-neo
Polylinker: CMV-KpnI-Sacl-BamHI-hNav β1-NotI-IRES-hyg

hNav1.8 Sequence (AF117907)
hNav β1 Sequence (NM_001037)
REFERENCES


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