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# Data Sheet

## Nav1.8/β2 – HEK293 Cell Line
## Catalog #: 60521
## Lot #: 160923

### Description
Stable recombinant HEK293 cell line expressing tetracycline-inducible human Nav1.8 (Genbank #NP_006505.2) fused to Green Fluorescent Protein (GFP) [Ex. ~395 nm, 475 nm; em ~510 nm] and the human sodium channel beta 2 subunit (Genbank #NM_004588; SCN2B). Nav1.8 is also known as tetrodotoxin-resistant voltage-gated sodium channel type X (SCN10A).

### Background
Nav1.8 is a voltage gated type X, alpha subunit sodium channel which in humans is encoded by the SCN10A gene. It is expressed in nociceptors and has been proposed as a target for the development of new analgesics. Mice deficient in Nav1.8 have deficits in sensing inflammatory pain (initiated by tissue damage/inflammation) and visceral pain (initiated by damage or injury to internal organs) but not neuropathic pain. Native sodium channels are complexes composed of the pore-forming α subunit and an auxiliary β subunit. The sodium channel beta 2 subunit, encoded by the SCN2B gene, increases current amplitude and surface expression of sodium ion channels.

### Sequence
A synthetic codon-optimized DNA sequence encoding human Nav1.8 (SCN10A gene) with C-terminal GFP-tag and C-terminal Streptavidin-Binding Peptide (SBP)-tag, and the SCN2B gene encoding human β2 subunit with C-terminal FLAG-tag are stably integrated in tetracycline-inducible HEK293 cells.

### Applications
- Drug compound screening
- Functional assays
- Efficient antigen for mouse immunization

### Format
Each vial contains ~1 X $10^6$ cells in 10% DMSO solution

### Host cell
HEK293 cells, tetracycline-inducible

### Mycoplasma testing
The cell line is confirmed for absence of *Mycoplasma* species using the PCR-based VenorGeM® Mycoplasma Detection kit (Sigma Aldrich).

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**Recommended Storage**
Immediately upon receipt, store in liquid nitrogen.

**Propagation Medium and Culture Conditions**

**Thaw Medium 4 (BPS Cat. #60181):** DMEM/F12 (1:1) (Hyclone # SH30271.01) supplemented with 10% FBS (Life Technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone # SV30010)

**Growth Medium 4B (BPS Cat. #79554) :** Thaw Medium 4 (BPS Cat. #60181) plus 10 μg/ml Blasticidin (Life Technologies # R210-01), 200 μg /ml Zeocin (Invivogen # ant-zn-1p), and 100 μg/ml Hygromycin B (Hyclone #SV30070.01)

Cells should be grown at 37°C with 5% $CO_2$ using complete growth medium (Thaw Medium 4, Blasticidin, Zeocin, and Hygromycin B)

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 4 **(no Blasticidin, Zeocin, and Hygromycin)**, spin down the cells, and resuspend cells in pre-warmed Thaw Medium 4 **(no Blasticidin, Zeocin, and Hygromycin)**. Transfer resuspended cells to a T25 flask and culture in 37°C $CO_2$ incubator. At first passage, switch to Growth Medium 4B **(contains Blasticidin, Zeocin and Hygromycin B)**. Cells should be split before they reach complete confluence.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA, add complete growth medium and transfer to a centrifuge tube. Spin down cells, resuspend cells in complete growth medium and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ratio: 1:20, twice per week.

**Induction of the target protein expression**
Induce cells in DMEM/F12 (1:1), 10% FBS, 1% Penicillin Streptomycin, 1 μg/ml Doxycycline (MP Biomedicals #0219504401) and 3 mM Na butyrate (Acros Organics #263190250) for 24 hours prior to cell harvesting or assay. Cells must be induced with doxycycline and Na-butyrate for ~24 hr to express the Nav1.8.

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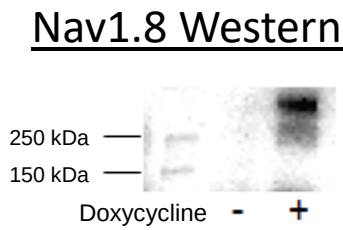
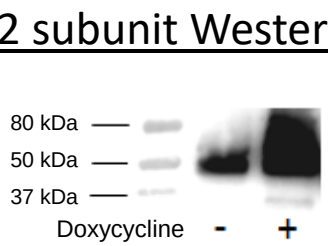
**Figure 1. Western Blot of the NaV1.8 and β2 expressing the Nav1.8/β2-HEK293 stable cell line.**

A) Western blot of tetracycline-induced Nav1.8-GFP, probed with anti-GFP antibody.




B) Western for β2 subunit-FLAG (constitutively expressed), probed with anti-FLAG antibody.




**References**

1. Catterall, W.A. *et al. Pharmacol. Rev.* **57** (4): 397–409 (2005).
2. Muzny, D.M., *et al. Nature* **440:**1194-1198 (2006).
3. Wilson, D.S., *et al. Protein Expression and Purification* **23** (3): 440–446 (2001).

**License Disclosure**

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