WEST BIOSCIENCE

9891 Irvine Center Dr. Suite 200
Irvine, CA 92618
United States
Tel: 1.800.831.1518
Fax: 1.800.831.1518
Email: sale@westbioscience.com

Data Sheet

Nav1.8/β2 – HEK293 Cell Line
Catalog #: w70532
Lot #: 140821-1

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).

WEST BIOSCIENCE

9891 Irvine Center Dr. Suite 200
Irvine, CA 92618
United States
Tel: 1.800.831.1518
Fax: 1.800.831.1518
Email: sale@westbioscience.com

Recommended Storage
Immediately upon receipt, store in liquid nitrogen.

Propagation Medium and Culture Conditions
Cells should be grown at 37°C with 5% CO_2 using DMEM/F12 (1:1) (Hyclone # SH30271.01) supplemented with 10% FBS (Life Technologies #26140-079), 1% Penicillin Streptomycin (Hyclone #SV30010.01), 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)

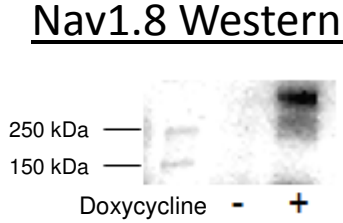
It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of growth medium without Blasticidin, Zeocin and Hygromycin B, spin down the cells, and resuspend cells in pre-warmed growth medium without Blasticidin, Zeocin and Hygromycin B. Transfer resuspended cells to a T25 flask and culture in 37°C CO_2 incubator. At first passage, switch to growth medium containing 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.

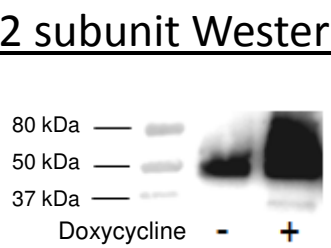
Figure 1. Western Blot of the NaV1.8 and β2 expressing the Nav1.8/β2-HEK293 stable cell line.

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



9891 Irvine Center Dr. Suite 200
Irvine, CA 92618
United States
Tel: 1.800.831.1518
Fax: 1.800.831.1518
Email: sale@westbioscience.com

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

Purchase of this cell line grants you with a 10-year license to use this cell line in your immediate laboratory, for research use only. This license does not permit you to share, distribute, sell, sublicense, or otherwise make the cell line available for use to other laboratories, departments, research institutions, hospitals, universities, or biotech companies. The license does not permit the use of this cell line in humans or for therapeutic or drug use. Inappropriate use or distribution of this cell line will result in revocation of the license and result in an immediate cease of sales and distribution of WestBio products to your laboratory. Publications using this cell line should reference West Bioscience, Inc., San Diego.