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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 



6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

Nav1.8/ β 2 – 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 β 2 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 $\sim 1 \times 10^6$ 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 Blastidicin (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% CO₂ using complete growth medium (Thaw Medium 4, Blastidicin, 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 Blastidicin, Zeocin, and Hygromycin**), spin down the cells, and resuspend cells in pre-warmed Thaw Medium 4 (**no Blastidicin, Zeocin, and Hygromycin**). Transfer resuspended cells to a T25 flask and culture in 37°C CO₂ incubator. At first passage, switch to Growth Medium 4B (**contains Blastidicin, 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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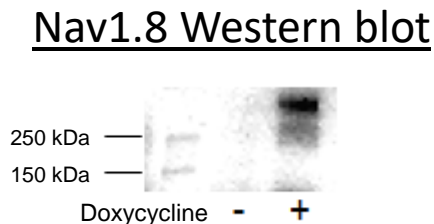
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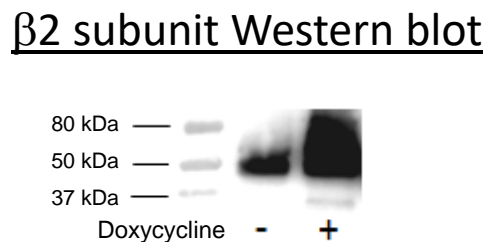
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Figure 1. Western Blot of the Nav1.8 and β 2 expressing the Nav1.8/ β 2-HEK293 stable cell line.

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



B) Western for β 2 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).

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