Description

Stable recombinant HEK293 cell line engineered for doxycycline-inducible expression of human Nav1.7 (Genbank #Q15858) fused at the C-terminus to Green Fluorescent Protein [Ex. ~395 nm, 475 nm; em ~510 nm] and with C-terminal Streptavidin-Binding Peptide (SBP) fusion. Nav1.7 is a tetrodotoxin-sensitive voltage-gated sodium channel type IX subunit alpha (SCN9A).

Background

Nav1.7 is a voltage-gated sodium ion channel that in humans is encoded by the SCN9A gene. It is usually expressed at high levels in two types of neurons, the nociceptive neurons at dorsal root ganglion and trigeminal ganglion, and sympathetic ganglion neurons, which are part of the autonomic (involuntary) nervous system.

Nav1.7 is present at the endings of pain-sensing nerves, the nociceptors, close to the region where the impulse is initiated. The Nav1.7 channel produces a rapidly activating and inactivating current which is sensitive to the level of tetrodotoxin. Knockout mice that lack Nav1.7 in nociceptors showed reduced response to inflammatory pain [1].

Application

- Drug compound screening
- Functional assays

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of cell freezing medium (BPS Bioscience #79796)

Parental Cell Line

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 4	BPS Bioscience #60181
Growth Medium 4A	BPS Bioscience #79535

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages. Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 4 (BPS Bioscience #60181):
DMEM/F12 (1:1) supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 4A (BPS Bioscience #79535):
DMEM/F12 (1:1) supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 10 μg/ml Blasticidin, and 200 μg /ml Zeocin.

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 4 **(no Blasticidin or Zeocin)**.
   Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.
2. Immediately spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 4 **(no Blasticidin or Zeocin)**.
3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.
4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 4 **(no Blasticidin or Zeocin)** and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.
5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium **4A (contains Blasticidin and Zeocin)**.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 4A and transfer to a tube. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium **4A (contains no Blasticidin and Zeocin)**. Seed into new culture vessels at the desired sub-cultivation ratio of 1:10 to 1:15 twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 4A and count the cells.
3. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 4°C Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell aliquots into cryogenic vials. Place the vials in an insulated container for slow cooling and store at -80°C overnight.
5. Transfer the vials to liquid nitrogen the next day for storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

A. Validation Data

Induction of the target protein expression

To express Nav1.7, cells are induced with DMEM/F12 50/50 supplemented with 10% FBS, 1% Penicillin Streptomycin, 1 µg/ml Doxycycline (Biochemika #44577) and 3 mM Na butyrate (Acros Organics #263190250) for 24 hours prior to cell harvesting or assay.

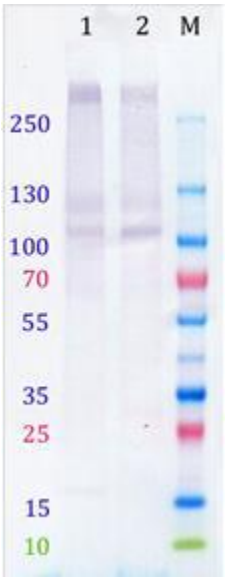

Figure 1. Western Blot of the Nav1.7 expressing cells.
Western Blot of HEK293 Nav1.7 cells (Lanes 1, 2) stained with anti-Sodium channel Nav1.7, clone N68/6 (Millipore #MABN41) followed by Alkaline Phosphatase-conjugated Anti-mouse IgG (Rockland Immunochemicals #610-1502). M: molecular weight marker.

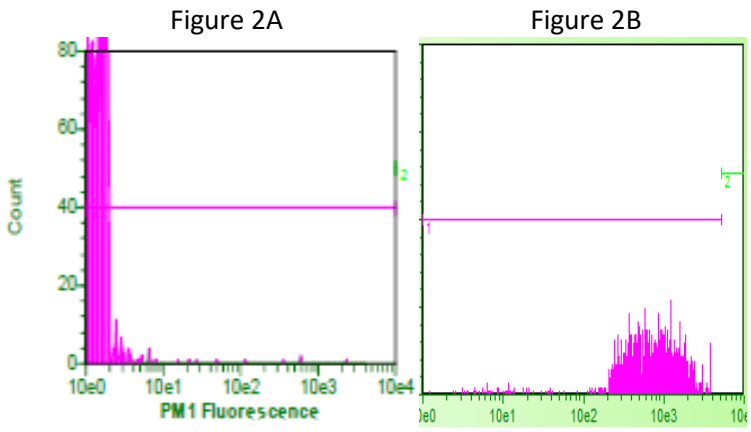

Figure 2. Cell Expression Profile.
Nav1.7 expression measured by flow cytometry using intracellular staining of saponin-permeabilized cells with anti-Nav1.7 clone 68/6 monoclonal antibody and PE-labeled anti-mouse. Figure 2A: HEK293 parental cells, Figure 2B: Nav1.7-expressing HEK293 cells (BPS Bioscience #60507) stained.

Sequence
A synthetic codon-optimized DNA sequence encoding human Nav1.7 protein [2] with C-terminal Green Fluorescent Protein (GFP) and C-terminal Streptavidin-Binding Peptide (SBP) [3] tag is stably integrated in tetracycline-inducible HEK293 cells.

Sequence of theNav1.7 Tag:
EGGGGSMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHM
KQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKV
NFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYKVEGLVPRGSG
SLVPRGSSAKETAAAKFERQHMDSGATETSQVAPAGAAAMDEKTTGWRGGHVVEGLAGELEQLRARLEHHPQGQREP

References

1. Catterall, W.A. *Cell and Developmental Biology* **16**: 521–555 (2000).
2. Choi, J.S., *et al. Neurology* **67**:1563-1567 (2006).
3. Li, Y., *el al. Protein Science* **20** (1): 140–149 (2011).

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
Nav1.8/β2 - HEK293 Recombinant Cell Line	60521	2 vials