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Data Sheet
Nav1.7 – HEK 293 Cell line
Catalog #: w70518

Description

Stable recombinant HEK293 cell line expressing human Nav1.7 (Genbank # Q15858) fused to Green Fluorescent Protein [Ex. ~395 nm, 475 nm; em ~510 nm]. 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].

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.

Applications

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

Format

Each vial contains 2 X 10^6 cells in 1 ml of 10% DMSO

Host cell

HEK293 cells, tetracycline-inducible

Recommended Storage

Immediately upon receipt, store in liquid nitrogen.

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MW

The calculated molecular weight is 242 kDa.

Stability

The cell line has been demonstrated to be stable for at least seven continuous passages. For optimal results, it is recommended to use the cells prior to the 7th passage. Upon receipt, amplify the cells in culture and make several frozen aliquots for future use.

Propagation Medium and Culture Conditions

DMEM/F12 50/50, 10% FBS, 1% Penicillin Streptomycin, 10 µg/ml Blasticidin, 0.2 mg/ml Zeocin.

Cells should be grown at 37° 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), and 200 µg /ml Zeocin (invivogen # ant-zn-1p).

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 and Zeocin, spin down the cells, and resuspend cells in pre-warmed growth medium without Blasticidin and Zeocin. Transfer resuspended cells to a T25 flask and culture in 37°C CO_2 incubator. At first passage switch to growth medium containing Blasticidin and Zeocin. 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.

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.

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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, Cat.No. MABN41) with follow staining with Alkaline Phosphatase conjugated Anti-mouse IgG (Rockland Immunochemicals, Cat.No. 610-1502). M: molecular weight marker.

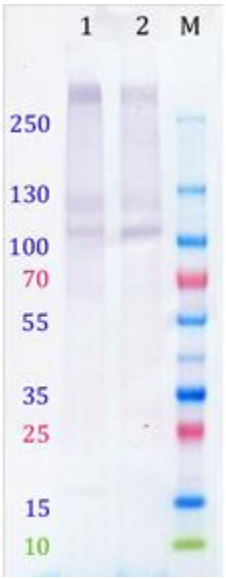


Figure 2. Cell Expression Profile

Figure 2a

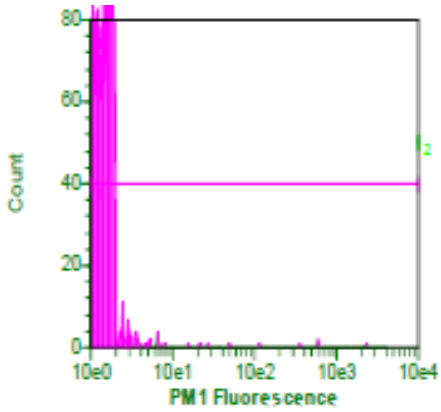
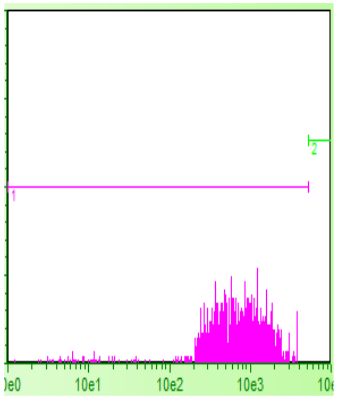


Figure 2b



NaV1.7 expression on the cell surface, measured by flow cytometry (FACS)
Figure 2a: HEK293 Host cells, Figure 2b: NaV1.7 expressing HEK293 cells (#w70518) stained with anti-NaV 1.7 clone 68/6 monoclonal and PE-labeled anti-mouse

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References

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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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