abcam

Product datasheet

Human NEFM (160 kD Neurofilament Medium) knockout HEK293T cell line ab266741

4 Images

Overview

Product name	Human NEFM (160 kD Neurofilament Medium) knockout HEK293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 2 and Insertion of the selection cassette in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**Recommended control:** Human wild-type HEK293T cell line (ab255449). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM (High Glucose) + 10% FBS **Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80ºC. Storage at -80ºC may result in loss of viability. 1. Thaw the vial in 37ºC water bath approximately 1-2 minutes. 2. Transfer the cell suspension (0.8 ml) to a 15 ml/50 ml conical sterile polypropylene centrifuge tube containing 8.4 ml pre-warmed **culture medium**, wash vial with an additional 0.8 ml **culture medium** (total volume 10 ml) to collect remaining cells, and centrifuge at 201 *x g* (rcf) for 5 minutes at room temperature. 10 ml represents minimum recommended dilution. 20 ml represents maximum recommended dilution. 3. Resuspend the cell pellet in 5 ml pre-warmed **culture medium** and count using a haemocytometer (Click here to view haemocytometer protocol) or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $2x10^4$ cells/cm^2. This should allow for confluency within 48 hours. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37ºC incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:**

All seeding densities should be based on cell counts gained by established methods.
A guide seeding density of $2x10^4$ cells/cm^2 is recommended for confluency (80-90% confluence) within 48 hours.
A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.
Cells should be passaged when they have achieved 80-90% confluence.
Click here to view the Mammalian cell tissue culture protocol

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Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Viability	~90%
Adherent /Suspension	Adherent
Tissue	Kidney
Cell type	epithelial
STR Analysis	Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% DMSO, 2% Cellulose, methyl ether

Target

Relevance	Neurofilaments are the 10nm or intermediate filament proteins found specifically in neurons, and are composed predominantly of three major proteins called neurofilament light (NF-L), neurofilament medium (NF-M) and neurofilament heavy (NF-H). Neurofilament medium runs on SDS-PAGE gels in the range 145-170 kDa, with some variation in different species. Antibodies to this protein are useful to identify neurons and their processes in tissue sections and in tissue culture. Neurofilament medium can also be useful in studies of neurofilament accumulations seen in many neurological diseases, such as Lou Gehrig's disease or Alzheimer's disease.
Cellular localization	Cytoplasm

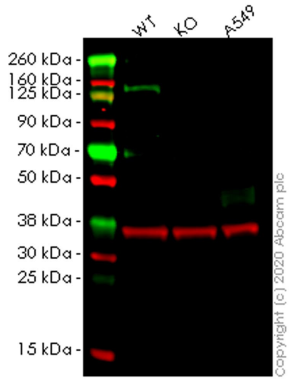
Applications

Our Abpromise guarantee covers the use of **ab266741** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 102 kDa.

Images



Western blot - Human NEFM (160 kD Neurofilament Medium) knockout HEK293T cell line (ab266741)

All lanes : Anti-160 kD Neurofilament Medium antibody [NF-09] - Neuronal Marker (ab7794) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate
Lane 2 : NEFM knockout HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate
Lane 3 : A549 (Human lung carcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) at 1/10000 dilution

Predicted band size: 102 kDa
Observed band size: 150 kDa
why is the actual band size different from the predicted?

Lanes 1-3: Merged signal (red and green). Green - ab7794 observed at 150 kDa. Red - loading control ab181602 observed at 36 kDa.

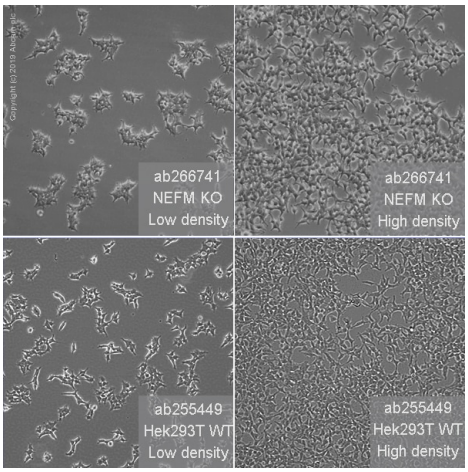
ab7794 Anti-160 kD Neurofilament Medium antibody [NF-09] was shown to specifically react with 160 kD Neurofilament Medium in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab266741 (knockout cell lysate ab257103) was used. Wild-type and 160 kD Neurofilament Medium knockout samples were subjected to SDS-PAGE. ab7794 and Anti-GAPDH antibody[EPR16891] - Loading Control (ab181602) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) and Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Allele-1: 1 bp insertion in exon 2

Sanger Sequencing - Human NEFM knockout HEK293T cell line (ab266741)

Allele-2: Insertion of the selection cassette in exon 2.

Sanger Sequencing - Human NEFM knockout HEK293T cell line (ab266741)



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