abcam

Product datasheet

Human NEFM (160 kD Neurofilament Medium) knockout HEK-293T cell line ab266741

4 图像

概述

产品名称	人NEFM (160 kD Neurofilament Medium) knockout HEK-293T 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)
经测试应用	**适用于:** WB
Biosafety level	2
常规说明	**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 -80°C. Storage at -80°C may result in loss of viability. 1. Thaw the vial in 37°C water bath for 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 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. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C 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.
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.
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We will provide viable cells that proliferate on revival.

性能

Number of cells	1 x 10^6 cells/vial, 1 mL
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
存放说明	Shipped on Dry Ice. Store in liquid nitrogen.
存储溶液	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

靶标

功能	Neurofilaments usually contain three intermediate filament proteins: L, M, and H which are involved in the maintenance of neuronal caliber.
序列相似性	Belongs to the intermediate filament family.
翻译后修饰	There are a number of repeats of the tripeptide K-S-P, NFM is phosphorylated on a number of the serines in this motif. It is thought that phosphorylation of NFM results in the formation of interfilament cross bridges that are important in the maintenance of axonal caliber. Phosphorylation seems to play a major role in the functioning of the larger neurofilament polypeptides (NF-M and NF-H), the levels of phosphorylation being altered developmentally and coincidentally with a change in the neurofilament function. Phosphorylated in the head and rod regions by the PKC kinase PKN1, leading to the inhibition of polymerization.

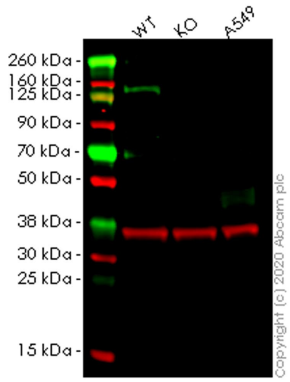
应用

The Abpromise guarantee **Abpromise™承诺保证使用**ab266741**于以下的**经测试应用

"应用说明"**部分 下显示的**仅为**推荐的起始稀释度**;实际**最佳的稀释度**/浓**度应由使用者检定。**

应用	Ab评论	说明
WB		Use at an assay dependent concentration. Predicted molecular weight: 102 kDa.

图片



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

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

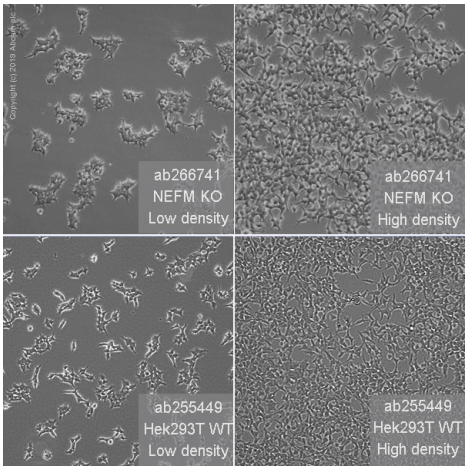
ab7794 Anti-160 kD Neurofilament Medium antibody [NF-09] was shown to specifically react with 160 kD Neurofilament Medium in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab266741 (knockout cell lysate **ab257103**) was used. Wild-type and 160 kD Neurofilament Medium knockout 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.

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

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.



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