abcam

Product datasheet

Human NONO (nmt55 / p54nrb) knockout HEK-293T cell line ab266244

1 References 3 Images

Overview

Product name	Human NONO (nmt55 / p54nrb) knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 17 bp deletion in exon 2 and 25 bp deletion 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 -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.
This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.

We will provide viable cells that proliferate on revival.

Properties

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
Antibiotic resistance	Puromycin 1.00µg/ml
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

Function	DNA- and RNA binding protein, involved in several nuclear processes. Binds the conventional octamer sequence in double stranded DNA. Also binds single-stranded DNA and RNA at a site independent of the duplex site (By similarity). Involved in pre-mRNA splicing, probably as an heterodimer with SFPQ. Interacts with U5 snRNA, probably by binding to a purine-rich sequence located on the 3' side of U5 snRNA stem 1b. The SFPQ-NONO heteromer associated with MATR3 may play a role in nuclear retention of defective RNAs. The SFPQ-NONO heteromer may be involved in DNA unwinding by modulating the function of topoisomerase I/TOP1. The SFPQ-NONO heteromer may be involved in DNA nonhomologous end joining (NHEJ) required for double-strand break repair and V(D)J recombination and may stabilize paired DNA ends. In vitro, the complex strongly stimulates DNA end joining, binds directly to the DNA substrates and cooperates with the Ku70/G22P1-Ku80/XRCC5 (Ku) dimer to establish a functional preligation complex. Nono is involved in transcriptional regulation. The SFPQ-NONO-NR5A1 complex binds to the CYP17 promoter and regulates basal and cAMP-dependent transcriptional avtivity. NONO binds to an enhancer element in long terminal repeats of endogenous intracisternal A particles (IAPs) and activates transcription.
Tissue specificity	Heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. Also found in a number of breast tumor cell lines.
Involvement in disease	Note=A chromosomal aberration involving NONO may be a cause of papillary renal cell carcinoma (PRCC). Translocation t(X;X)(p11.2;q13.1) with TFE3.
Sequence similarities	Contains 2 RRM (RNA recognition motif) domains.
Post-translational modifications	The N-terminus is blocked.

Cellular localization Nucleus.

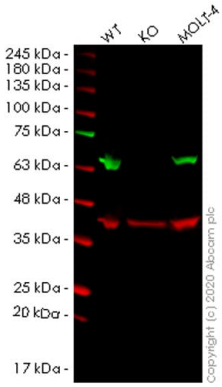
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab266244 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: 54 kDa.

Images



Western blot - Human NONO knockout HEK293T cell line (ab266244)

All lanes : Anti-nmt55 / p54nrb antibody [EPR5270] (**ab133574**) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : NONO knockout HEK293T cell lysate
Lane 3 : MOLT-4 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 54 kDa
Observed band size: 63 kDa

Lanes 1-3: Merged signal (red and green). Green - **ab133574** observed at 63 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab133574 Anti-nmt55 / p54nrb antibody [EPR5270] was shown to specifically react with nmt55 / p54nrb in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266244 (knockout cell lysate **ab257160**) was used. Wild-type and nmt55 / p54nrb knockout samples were subjected to SDS-PAGE. **ab133574** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) 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® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed

(**ab216776**) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.

Sanger Sequencing - Human NONO knockout HEK293T cell line (ab266244)

Allele-1: 25 bp deletion in exon2

Sanger Sequencing - Human NONO knockout HEK293T cell line (ab266244)

Allele-2: 17 bp deletion in exon 2.

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