

Human NT5E knockout A-431 cell line ab261895

4 Images

Overview

Product name	Human NT5E knockout A-431 cell line
Parental Cell Line	A431
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 5 bp deletion, 2 bp deletion; Frameshift = 99.2%
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS), Western Blot (WB)
Tested applications	Suitable for: WB, Next Generation Sequencing
Biosafety level	1
General notes	<p>Recommended control: Human wild-type A-431 cell line (ab263975). 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.</p> <p>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: DMEM (High Glucose) + 10% FBS</p> <p>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.</p> <ol style="list-style-type: none"> 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 2×10^4 cells/cm². 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% CO₂. Cultures should be monitored daily. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of 2×10^4 cells/cm² is recommended.</p> <p>A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.</p>

Cells should be passaged when they have achieved 80-90% confluence.
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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	Skin
Cell type	epithelial
Disease	Epidermoid Carcinoma
Gender	Female
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

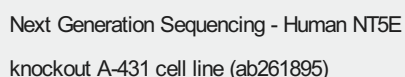
Function	Hydrolyzes extracellular nucleotides into membrane permeable nucleosides.
Involvement in disease	Defects in NT5E are the cause of calcification of joints and arteries (CAJA) [MIM:211800]. A condition characterized by adult-onset calcification of the lower extremity arteries, including the iliac, femoral and tibial arteries, and hand and foot capsule joints. Age of onset has been reported as early as the second decade of life, usually involving intense joint pain or calcification in the hands.
Sequence similarities	Belongs to the 5'-nucleotidase family.
Cellular localization	Cell membrane.

Applications

The Abpromise guarantee Our [Abpromise guarantee](#) covers the use of ab261895 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.
Next Generation Sequencing		Use at an assay dependent concentration.

Images



All lanes : Anti-CD73 antibody [4G6E3] (**ab202122**) at 1/1000 dilution

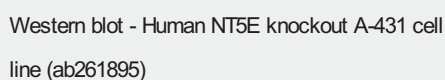
Lane 1 : Wild-type A-431 whole cell lysate

Lane 2 : NT5E knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 3 : A375 (Human malignant melanoma cell line) whole cell lysate

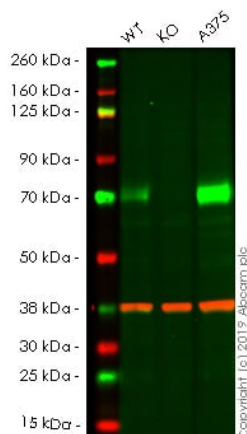
Lysates/proteins at 20 μ g per lane.

Performed under reducing conditions.



Lanes 1 - 3: Merged signal (red and green). Green - **ab202122** observed at 63 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

ab202122 was shown to recognize NT5E in wild-type A-431 cells as signal was lost at the expected MW in NT5E knockout cell line ab261895 (knockout cell lysate **ab261704**). Additional cross-reactive bands were observed in the wild-type and knockout samples. Wild-type and NT5E knockout samples were subjected to SDS-PAGE. Ab202122 and **ab181602** (Rabbit anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed **ab216772** and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed **ab216777** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human NT5E knockout A-431 cell line (ab261895)

All lanes : Anti-CD73 antibody [EPR6114] ([ab133582](#)) at 1/1000 dilution

Lane 1 : Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 2 : NT5E knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 3 : A-375 (Human malignant melanoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Lanes 1 - 3: Merged signal (red and green). Green - [ab133582](#) observed at 63 kDa. Red - loading control, [ab8245](#), observed at 37 kDa.

[ab133582](#) was shown to recognize NT5E in wild-type A-431 cells as signal was lost at the expected MW in NT5E knockout cell line ab261895 (knockout cell lysate [ab261704](#)). Additional cross-reactive bands were observed in the wild-type and knockout samples. Wild-type and NT5E knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% milk. Ab133582 and [ab8245](#) (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed [ab216773](#) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed [ab216776](#) secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

x = 5 bp deletion, 2 bp deletion

CCCTGCGCTACGATGCCATGCTAAGACCCGAGCC Reference

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CCCTGCGCTACG-----ATGCTAAGACCCGAGCC Deletion, 6873 reads, 39.77%

CCCTGCGCTACGATGCCATGCTAAGACCCGAGCC Reference

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CCCTGCGCTACGATG--ATGCTAAGACCCGAGCC Deletion, 6765 reads, 39.14%

Next Generation Sequencing - Human NT5E

knockout A-431 cell line (ab261895)

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