abcam

Product datasheet

Human PXN (Paxillin) knockout A-431 cell line ab261892

画像数 5

製品の概要

製品名	Human PXN (Paxillin) knockout A-431 cell line
Parental Cell Line	A431
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 7 bp deletion, 1 bp insertion; Frameshift = 99.7%
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS), Western Blot (WB)
アプリケーション	**適用あり:** WB, Next Generation Sequencing
Biosafety level	1
特記事項	**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. **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.

製品の特性

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
保存方法	Shipped on Dry Ice. Store in liquid nitrogen.
バッファー	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

ターゲット情報

機能	Cytoskeletal protein involved in actin-membrane attachment at sites of cell adhesion to the extracellular matrix (focal adhesion).
配列類似性	Belongs to the paxillin family. Contains 4 LIM zinc-binding domains.
翻訳後修飾	Phosphorylated on tyrosine residues during integrin-mediated cell adhesion, embryonic development, fibroblast transformation and following stimulation of cells by mitogens.
細胞内局在	Cytoplasm > cytoskeleton. Cell junction > focal adhesion.

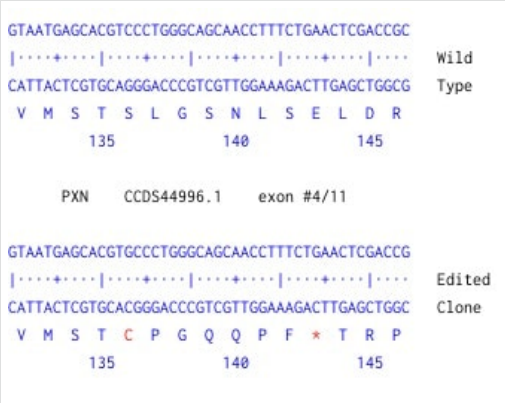
アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab261892の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

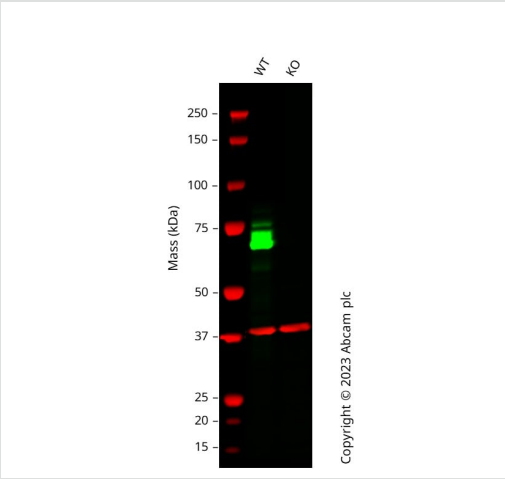
アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration.
Next Generation Sequencing		Use at an assay dependent concentration.

画像



Next Generation Sequencing - Human PXN (Paxillin) knockout A-431 cell line (ab261892)

1 bp insertion after Thr135 of the WT protein



Western blot - Human PXN (Paxillin) knockout A-431 cell line (ab261892)

All lanes : Anti-Paxillin antibody [Y113] (**ab32084**) at 1/1000 dilution

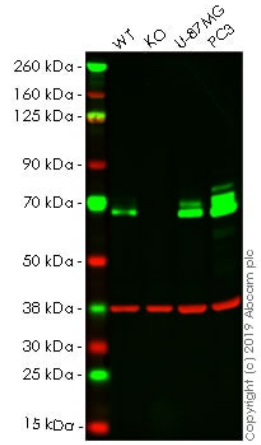
Lane 1 : Wild-type A431 cell lysate
Lane 2 : PXN knockout A431 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 70 kDa

Anti-PXN antibody [Y113] (**ab32084**) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab32084** was shown to bind specifically to PXN. A band was observed at 70 kDa in wild-type A431 cell lysates with no signal observed at this size in PXN knockout cell line ab261892 (knockout cell lysate **ab261701**). To generate this image, wild-type and PXN knockout A431 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween$®$ 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution



Western blot - Human PXN (Paxillin) knockout A-431 cell line (ab261892)

All lanes : Anti-Paxillin antibody [E228] (**ab32115**) at 1/10000 dilution

Lane 1 : Wild-type A431 whole cell lysate
Lane 2 : PXN knockout A431 whole cell lysate
Lane 3 : U-87 MG whole cell lysate
Lane 4 : PC-3 whole cell lysate

Lysates/proteins at 20 µg per lane.

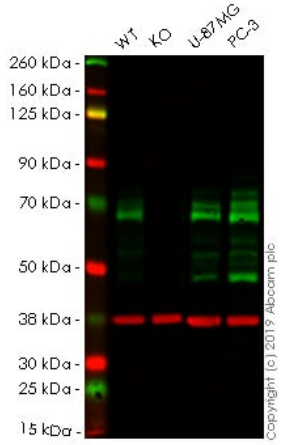
Performed under reducing conditions.

Observed band size: 65 kDa

Exposure time: 10 seconds

Lanes 1 - 4: Merged signal (red and green). Green - **ab32115** observed at 65 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab32115 was shown to specifically react with PXN in wild-type A-431 cells as signal was lost in PXN knockout cell line ab261892 (knockout cell lysate **ab261701**). Wild-type and PXN knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% milk. Ab32115 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/10000 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.



Western blot - Human PXN (Paxillin) knockout A-431 cell line (ab261892)

All lanes : Anti-Paxillin antibody [M107] (**ab23510**) at 1/1000 dilution

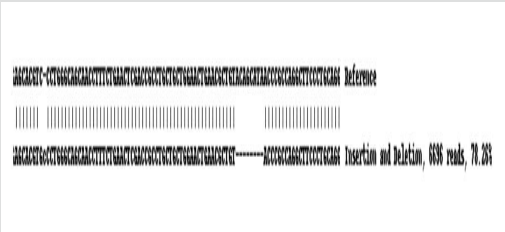
Lane 1 : Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 2 : PXN knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 3 : U-87 MG (Human glioblastoma-astrocytoma epithelial cell line) whole cell lysate
Lane 4 : PC3 (Human prostate adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Lanes 1 - 4: Merged signal (red and green). Green - **ab23510** observed at 65 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

ab23510 was shown to recognize PXN in wild-type A-431 cells as signal was lost at the expected MW in PXN knockout cell line ab261892 (knockout cell lysate **ab261701**). Additional cross-reactive bands were observed in the wild-type and knockout samples. Wild-type and PXN knockout samples were subjected to SDS-PAGE. Ab23510 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.



Next Generation Sequencing - Human PXN (Paxillin) knockout A-431 cell line (ab261892)

X = 7 bp deletion, 1 bp insertion

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