abcam

Product datasheet

Human MMP14 knockout A-431 cell line ab261890

4 Images

Overview

Product name	Human MMP14 knockout A-431 cell line
Parental Cell Line	A431
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 10 bp deletion; Frameshift = 99.9%
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Next Generation Sequencing (NGS), Western Blot (WB)
Tested applications	**Suitable for:** WB, ICC/IF, Next Generation Sequencing
Biosafety level	1
General notes	**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.
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	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	Seems to specifically activate progelatinase A. May thus trigger invasion by tumor cells by activating progelatinase A on the tumor cell surface. May be involved in actin cytoskeleton reorganization by cleaving PTK7.
Tissue specificity	Expressed in stromal cells of colon, breast, and head and neck. Expressed in lung tumors.
Sequence similarities	Belongs to the peptidase M10A family. Contains 4 hemopexin-like domains.
Domain	The conserved cysteine present in the cysteine-switch motif binds the catalytic zinc ion, thus inhibiting the enzyme. The dissociation of the cysteine from the zinc ion upon the activation-peptide release activates the enzyme.
Post-translational modifications	The precursor is cleaved by a furin endopeptidase.
Cellular localization	Membrane. Melanosome. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

Applications

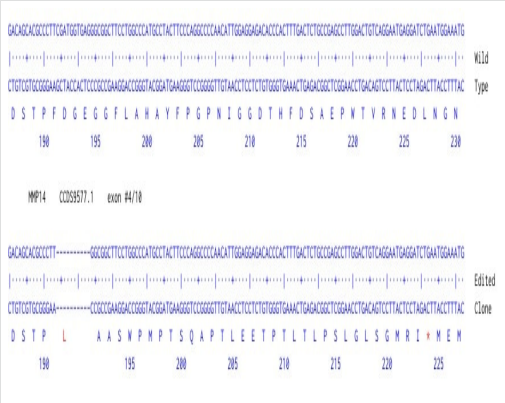
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab261890 in the following tested applications.

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.

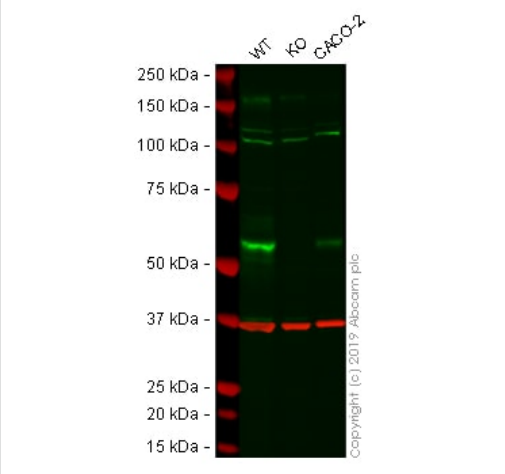
Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
Next Generation Sequencing		Use at an assay dependent concentration.

Images



Next Generation Sequencing - Human MMP14 knockout A-431 cell line (ab261890)

10 bp deletion after Pro190 of the WT protein



Western blot - Human MMP14 knockout A-431 cell line (ab261890)

All lanes : Anti-MMP14 antibody [EP1264Y] (**ab51074**) at 1/2000 dilution

Lane 1 : Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 2 : MMP14 knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 3 : Caco-2 (Human colorectal adenocarcinoma cell line) whole cell lysate

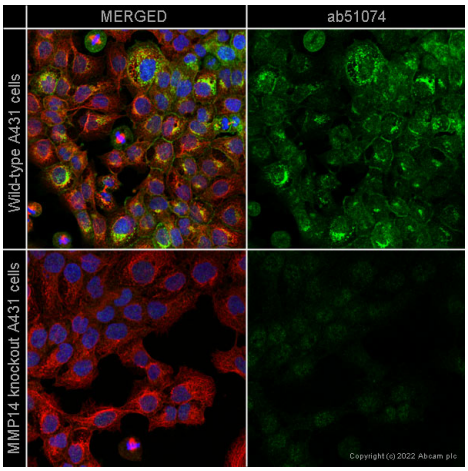
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Lanes 1 - 3: Merged signal (red and green). Green - **ab51074** observed at 54 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

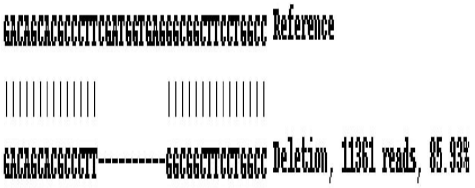
ab51074 was shown to react with MMP14 in wild-type A-431 cells in Western blot Loss of signal was observed when MMP14 knockout cell line ab261890 (knockout cell lysate **ab261699**) was

used. Wild-type A-431 and MMP14 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBS-T (0.1% Tween®) before incubation with **ab51074** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 2000 dilution and a 1 in 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 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Human MMP14 knockout A-431 cell line (ab261890)

ab51074 staining MMP14 in wild-type A431 cells (top panel) and MMP14 knockout A431 cells (bottom panel) (ab261890). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab51074** at 0.2µg/ml concentration and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Next Generation Sequencing - Human MMP14 knockout A-431 cell line (ab261890)

X = 10 bp deletion

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