abcam

Product datasheet

Human IL1RN (IL1 Receptor Antagonist) knockout A-431 cell line ab273379

5 Images

Overview

Product name	Human IL1RN (IL1 Receptor Antagonist) knockout A-431 cell line
Parental Cell Line	A431
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 49 bp deletion in exon 5.
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB, Sandwich ELISA, ICC/IF
Biosafety level	1
General notes	**Recommended control:** Human wild-type A-431 cell line (**ab275462**). **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** McCoY5a + 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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Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Viability	~80%
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

Relevance Interleukin 1 receptor antagonist inhibits the activities of interleukin 1 alpha and beta, and modulates a variety of interleukin 1 related immune and inflammatory responses. This gene and five other related cytokine genes form a cluster spanning approximately 400 kb on chromosome 2. A polymorphism of this gene is associated with increased risk of developing osteoporosis and gastric cancer. Four alternatively spliced transcript variants encoding differentt isoforms have been reported.

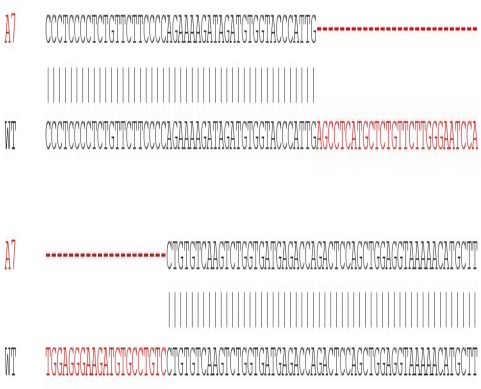
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab273379 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

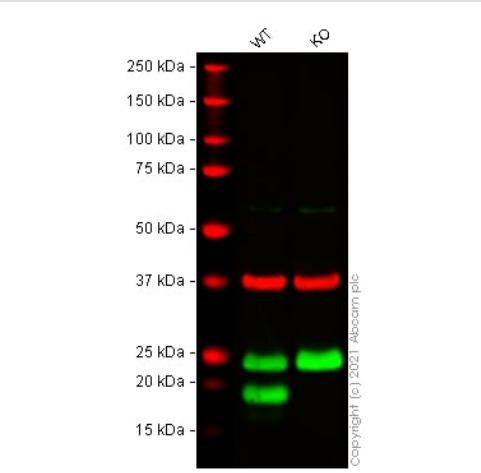
Application	Abreviews	Notes
WB		Use at an assay dependent concentration.
Sandwich ELISA		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.

Images



Sanger Sequencing - Human IL1RN knockout A-431 cell line (ab273379)

49 bp deletion in exon 5



Western blot - Human IL1RN knockout A-431 cell line (ab273379)

All lanes : Anti-IL-1RA antibody [EPR6483] (**ab124962**) at 1/10000 dilution

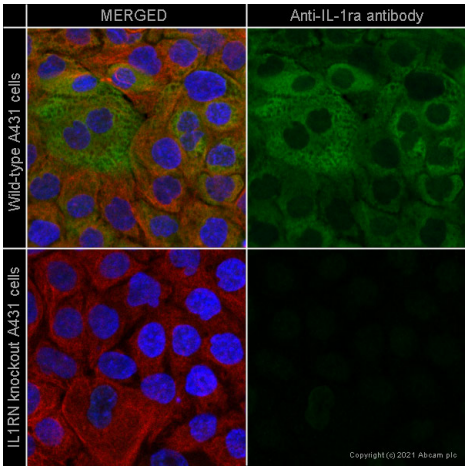
Lane 1 : Wild-type A431 cell lysate
Lane 2 : IL-1RA knockout A431 cell lysate

Performed under reducing conditions.

Observed band size: 19 kDa

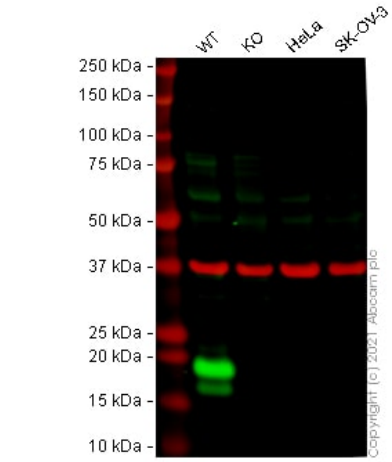
False colour image of Western blot: Anti-IL-1RA antibody [EPR6483] staining at 1/10000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab124962** was shown to bind specifically to IL-1RA. A band was observed at 19 kDa in wild-type A431 cell lysates with no signal observed at this size in IL1RN knockout cell line ab273379 (knockout cell lysate **ab275530**). To generate this image, wild-type and IL1RN 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$^{Â®}$ 20 (TBS-T) before incubation with primary antibodies overnight at 4 Â°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 (IRDye$^{Â®}$ 800CW) preabsorbed (**ab216773**) and

Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Immunocytochemistry - Human IL1RN knockout A-431 cell line (ab273379)

IL-1RA staining observed in wild-type A431 cells (top panel) and IL1RN knockout A431 cells (bottom panel). The cells were fixed with 100% methanol (5 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 an anti-IL-1ra antibody at 10µg/ml concentration (shown in green) and **ab195889** (Mouse monoclonal to alpha Tubulin - Alexa Fluor® 594) at 1/250 dilution (shown in red) overnight at 4°C. Nuclear DNA was labelled in blue with DAPI. Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Western blot - Human IL1RN knockout A-431 cell line (ab273379)

All lanes : Anti-IL-1ra antibody at 0.5 µg/ml

Lane 1 : Wild-type A431 cell lysate
Lane 2 : IL-1RA knockout A431 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : SK-OV-3 cell lysate

Lysates/proteins at 20 µg per lane.

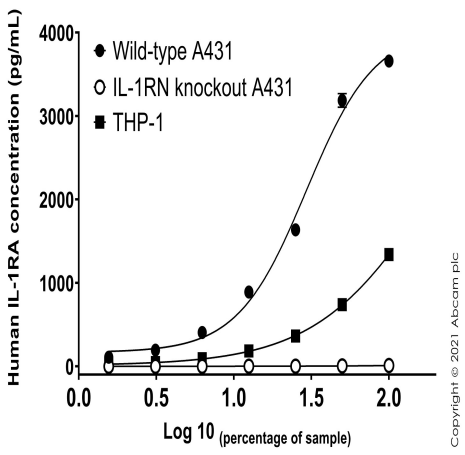
Performed under reducing conditions.

Observed band size: 18 kDa

Lanes 1 - 4: Merged signal (red and green). Green - Anti-IL-1ra antibody observed at 18 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

Anti-IL-1ra antibody was shown to react with IL-1ra in wild-type A431 cells in Western blot with loss of signal observed in IL-1RA knockout cell line ab273379 (IL-1RA knockout cell lysate **ab275530**). Wild-type A431 and IL-1RA knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with anti-IL-1ra antibody and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at 0.5 µg/ml and a 1 in 20000 dilution

respectively. Blots were incubated with Donkey anti-Goat IgG H&L (IRDye® 800CW) preabsorbed (**ab216775**) and Donkey anti-Mouse 680RD secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Sandwich ELISA - Human IL1RN knockout A-431 cell line (ab273379)

Human IL-1RA concentration was interpolated from the IL-1RA standard curve. Supernatants from cell culture samples were serially diluted and assessed by the Human IL-1ra ELISA Kit (**ab211650**). Wild-type A-431, IL1RN knockout A-431 (ab273379) and THP-1 cells were assessed in duplicate (n=2). Data are represented as the mean and error bars represent standard deviation.

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