abcam

Product datasheet

Human KRT13 knockout A-431 cell line ab269483

4 Images

Overview

Product name Human KRT13 knockout A-431 cell line

Parental Cell Line A431
Organism Human

Mutation description Knockout achieved by CRISPR/Cas9; X = 5 bp deletion; Frameshift: 100%

Passage number <20

Knockout validation Next Generation Sequencing (NGS), Western Blot (WB)

Tested applications Suitable for: WB

Biosafety level

General notesRecommended control: Human wild-type A-431 cell line (<u>ab263975</u>). 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: 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 2x10⁴ 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.

Subculture guidelines:

All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $2x10^4$ cells/cm² is recommended.

A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.

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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 10⁶ 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

Tissue specificity Expressed in some epidermal sweat gland ducts (at protein level) and in exocervix, esophagus

and placenta.

Involvement in disease Defects in KRT13 are a cause of white sponge nevus of cannon (WSN) [MIM:193900]. WSN is a

rare autosomal dominant disorder which predominantly affects non-cornified stratified squamous epithelia. Clinically, it is characterized by the presence of soft, white, and spongy plaques in the oral mucosa. The characteristic histopathologic features are epithelial thickening, parakeratosis, and vacuolization of the suprabasal layer of oral epithelial keratinocytes. Less frequently the

mucous membranes of the nose, esophagus, genitalia and rectum are involved.

Sequence similaritiesBelongs to the intermediate filament family.

Post-translational modifications

O-glycosylated; glycans consist of single N-acetylglucosamine residues.

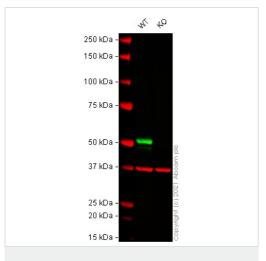
Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab269483 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. Detects a band of approximately 51 kDa (predicted molecular weight: 50 kDa).

Images



Western blot - Human KRT13 knockout A-431 cell line (ab269483)

All lanes : Anti-Cytokeratin 13 antibody [EPR3671] (<u>ab92551</u>) at 1/100000 dilution

Lane 1: Wild-type A431 cell lysate

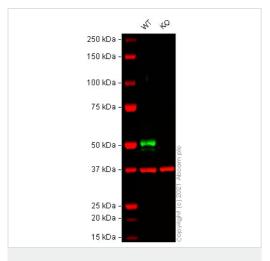
Lane 2: KRT13 knockout A431 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 50 kDa **Observed band size:** 51 kDa

False colour image of Western blot: Anti-Cytokeratin 13 antibody [EPR3671] staining at 1/100000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab92551 was shown to bind specifically to Cytokeratin 13. A band was observed at 51 kDa in wild-type A431 cell lysates with no signal observed at this size in Krt13 knockout cell line ab269483 (knockout cell lysate ab269647). To generate this image, wild-type and Krt13 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 (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Western blot - Human KRT13 knockout A-431 cell line (ab269483)

All lanes : Anti-Cytokeratin 13 antibody [AE8] ($\underline{ab16112}$) at 1 $\mu g/ml$

Lane 1: Wild-type A431 cell lysate

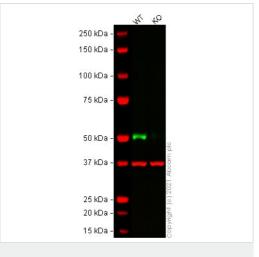
Lane 2: KRT13 knockout A431 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 50 kDa Observed band size: 51 kDa

False colour image of Western blot: Anti-Cytokeratin 13 antibody [AE8] staining at 1 µg/ml, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (ab181602) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab16112 was shown to bind specifically to Cytokeratin 13. A band was observed at 51 kDa in wild-type A431 cell lysates with no signal observed at this size in Krt13 knockout cell line ab269483 (knockout cell lysate ab269647). To generate this image, wild-type and Krt13 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-Mouse IgG H&L (IRDye® 800CW) preabsorbed (ab216772) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (ab216777) at 1/20000 dilution.



Western blot - Human KRT13 knockout A-431 cell line (ab269483)

All lanes : Anti-Cytokeratin 13 antibody [EPR3672] (<u>ab133340</u>) at 1/2000 dilution

Lane 1: Wild-type A431 cell lysate

Lane 2: KRT13 knockout A431 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 50 kDa **Observed band size:** 51 kDa

False colour image of Western blot: Anti-Cytokeratin 13 antibody [EPR3672] staining at 1/2000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab133340 was shown to bind specifically to Cytokeratin 13. A band was observed at 51 kDa in wild-type A431 cell lysates with no signal observed at this size in Krt13 knockout cell line ab269483 (knockout cell lysate ab269647). To generate this image, wild-type and Krt13 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 (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.

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Next Generation Sequencing - Human KRT13 knockout A-431 cell line (ab269483)

Knockout achieved by CRISPR/Cas9; X = 5 bp deletion; Frameshift: 100%

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