abcam

Product datasheet

Human KRT14 (Cytokeratin 14) knockout A-431 cell lysate ab261706

4 Images

Overview

Product name Human KRT14 (Cytokeratin 14) knockout A-431 cell lysate

Product overview Knockout cell lysate achieved by CRISPR/Cas9.

Parental Cell Line A431

Organism Human

Mutation description Knockout achieved by CRISPR/Cas9; X = 13 bp deletion; Frameshift = 99.9%

Passage number <20

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

Reconstitution notes

To use as WB control, resuspend the lyophilizate in 50 μL of LDS* Sample Buffer to have a final

concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M

DTT.

*Usage of SDS sample buffer is not recommended with these lyophilized lysates.

Notes

Lysate preparation: Our lysates are made using RIPA buffer to which we add a protease

inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are

prepared.

User storage instructions: Lyophilizate may be stored at 4°C. After reconstitution, store at -

20°C for short-term storage or -80°C for long-term storage.

Access thousands of knockout cell lysates, generated from commonly used cancer cell lines.

See here for more information on knockout cell lysates.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of

products that contain European Authorisation list (Annex XIV) substances.

It is the responsibility of our customers to check the necessity of application of REACH

Authorisation, and any other relevant authorisations, for their intended uses.

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.

Tested applications Suitable for: WB

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Properties

Storage instructions

Store at -80°C. Please refer to protocols.

Components	1 kit
ab280456 - Human KRT14 knockout A431 cell lysate	1 x 100µg
ab263973 - Human wild-type A-431 cell lysate	1 x 100µg

Cell type epithelial

Disease Epidermoid Carcinoma

Gender Female

Target

Function

The nonhelical tail domain is involved in promoting KRT5-KRT14 filaments to self-organize into large bundles and enhances the mechanical properties involved in resilience of keratin intermediate filaments in vitro.

Tissue specificity

Detected in the basal layer, lowered within the more apically located layers specifically in the stratum spinosum, stratum granulosum but is not detected in stratum corneum. Strongly expressed in the outer root sheath of anagen follicles but not in the germinative matrix, inner root sheath or hair. Found in keratinocytes surrounding the club hair during telogen.

Involvement in disease

Defects in KRT14 are a cause of epidermolysis bullosa simplex Dowling-Meara type (DM-EBS) [MIM:131760]. DM-EBS is a severe form of intraepidermal epidermolysis bullosa characterized by generalized herpetiform blistering, milia formation, dystrophic nails, and mucous membrane involvement.

Defects in KRT14 are a cause of epidermolysis bullosa simplex Weber-Cockayne type (WC-EBS) [MIM:131800]. WC-EBS is a form of intraepidermal epidermolysis bullosa characterized by blistering limited to palmar and plantar areas of the skin.

Defects in KRT14 are a cause of epidermolysis bullosa simplex Koebner type (K-EBS) [MIM:131900]. K-EBS is a form of intraepidermal epidermolysis bullosa characterized by generalized skin blistering. The phenotype is not fundamentally distinct from the Dowling-Meara type, although it is less severe.

Defects in KRT14 are the cause of epidermolysis bullosa simplex autosomal recessive (AREBS) [MIM:601001]. AREBS is an intraepidermal epidermolysis bullosa characterized by localized blistering on the dorsal, lateral and plantar surfaces of the feet.

Defects in KRT14 are the cause of Naegeli-Franceschetti-Jadassohn syndrome (NFJS) [MIM:161000]; also known as Naegeli syndrome. NFJS is a rare autosomal dominant form of ectodermal dysplasia. The cardinal features are absence of dermatoglyphics (fingerprints), reticular cutaneous hyperpigmentation (starting at about the age of 2 years without a preceding inflammatory stage), palmoplantar keratoderma, hypohidrosis with diminished sweat gland function and discomfort provoked by heat, nail dystrophy, and tooth enamel defects.

Defects in KRT14 are the cause of dermatopathia pigmentosa reticularis (DPR) [MIM:125595].

DPR is a rare ectodermal dysplasia characterized by lifelong persistent reticulate

hyperpigmentation, noncicatricial alopecia, and nail dystrophy.

Sequence similarities

Belongs to the intermediate filament family.

Cellular localization

Cytoplasm. Nucleus. Expressed in both as a filamentous pattern.

Applications

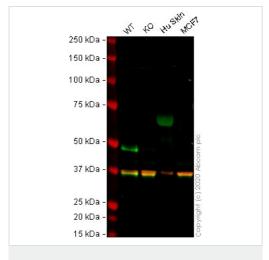
The Abpromise guarantee

Our Abpromise guarantee covers the use of ab261706 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. Predicted molecular weight: 52 kDa.

Images



Western blot - Human KRT14 (Cytokeratin 14) knockout A-431 cell lysate (ab261706) Lane 1: Wild-type A431 cell lysate 20 ug

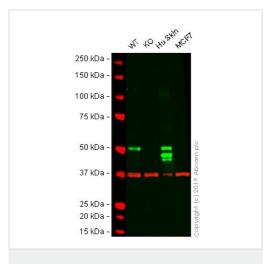
Lane 2: KRT14 knockout A431 cell lysate 20 ug

Lane 3: Human skin cell lysate 20 ug

Lane 4: MCF7 (Human breast adenocarcinoma cell line) whole cell lysate 20 ug

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab51054</u> observed at 49 kDa. Red - loading control, <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab51054 was shown to react with Cytokeratin 14 in wild-type A-431 cells in western blot Loss of signal was observed when KRT14 knockout cell line ab261897 (knockout cell lysate ab261706) was used. Wild-type A-431 and KRT14 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab51054 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human KRT14 (Cytokeratin 14) knockout A-431 cell lysate (ab261706)

Lane 1: Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate 20 ug

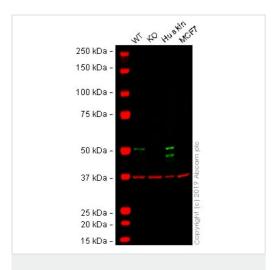
Lane 2: KRT14 knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate 20 ug

Lane 3: Human skin whole tissue lysate 20 ug

Lane 4: MCF7 (Human breast adenocarcinoma cell line) whole cell lysate 20 ug

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab197893</u> observed at 52 kDa. Red - loading control, <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab197893 was shown to react with KRT14 in wild-type A-431 cells in Western blot Loss of signal was observed when KRT14 knockout cell line ab261897 (knockout cell lysate ab261706) was used. Wild-type A-431 and KRT14 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab197893 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 50000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human KRT14 (Cytokeratin 14) knockout A-431 cell lysate (ab261706)

Lane 1: Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate 20 ug

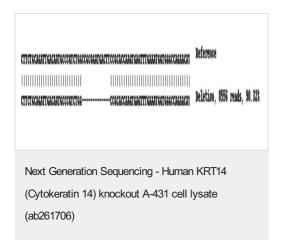
Lane 2: KRT14 knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate 20 ug

Lane 3: Human skin whole tissue lysate 20 ug

Lane 4: MCF7 (Human breast adenocarcinoma cell line) whole cell lysate 20 ug

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab119695</u> observed at 52 kDa. Red - loading control, <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab119695 was shown to react with KRT14 in wild-type A-431 cells in Western blot Loss of signal was observed when KRT14 knockout cell line ab261897 (knockout cell lysate ab261706) was used. Wild-type A-431 and KRT14 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab119695 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 93 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.



Knockout achieved by CRISPR/Cas9; X = 13 bp deletion; Frameshift = 99.9%

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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