abcam

Product datasheet

Human KRT14 (Cytokeratin 14) knockout A-431 cell line ab261897

11 图像

概述

产品名称	人KRT14 (Cytokeratin 14) knockout A-431 cell line
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	Immunocytochemistry (ICC), Next Generation Sequencing (NGS), Western Blot (WB)
经测试应用	**适用于:** WB, ICC/IF
Biosafety level	1
常规说明	**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.

性能

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
存放说明	Shipped on Dry Ice. Store in liquid nitrogen.
存储溶液	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

靶标

功能	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.
组织特异性	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.
疾病相关	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.
序列相似性	Belongs to the intermediate filament family.
细胞定位	Cytoplasm. Nucleus. Expressed in both as a filamentous pattern.

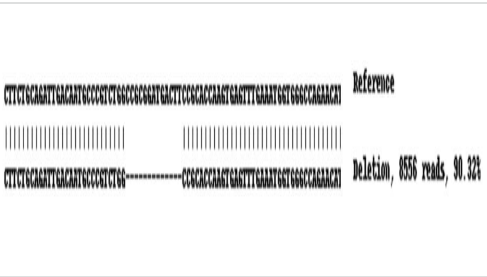
应用

The Abpromise guarantee **Abpromise™承诺保证使用**ab261897**于以下的**经测试应用

"应用说明"**部分 下显示的**仅为**推荐的起始稀释度**;实际**最佳的稀释度**/浓度应**由使用者检定。**

应用	Ab评论	说明
WB		Use at an assay dependent concentration. Predicted molecular weight: 52 kDa.
ICC/IF		Use at an assay dependent concentration.

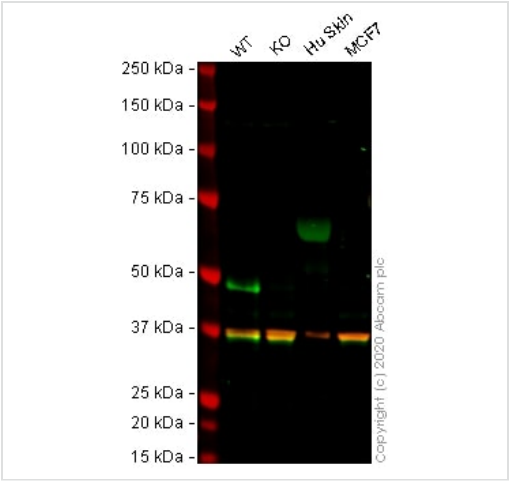
图片



Next Generation Sequencing - Human KRT14 (Cytokeratin 14) knockout A-431 cell line (ab261897)

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

Frameshift = 99.9%



Western blot - Human KRT14 (Cytokeratin 14) knockout A-431 cell line (ab261897)

All lanes : Anti-Cytokeratin 14 antibody [EP1612Y] (**ab51054**) at 1/10000 dilution

Lane 1 : Wild-type A431 cell lysate
Lane 2 : KRT14 knockout A431 cell lysate
Lane 3 : Human skin cell lysate
Lane 4 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

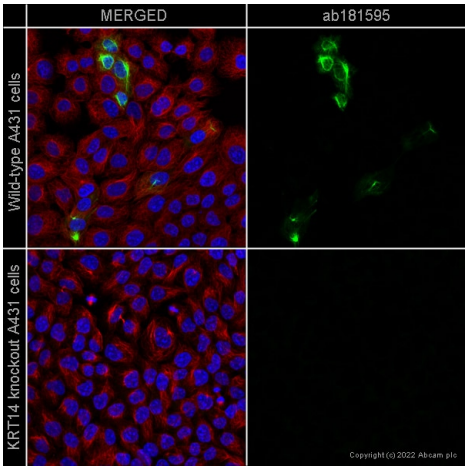
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 52 kDa
Observed band size: 49 kDa

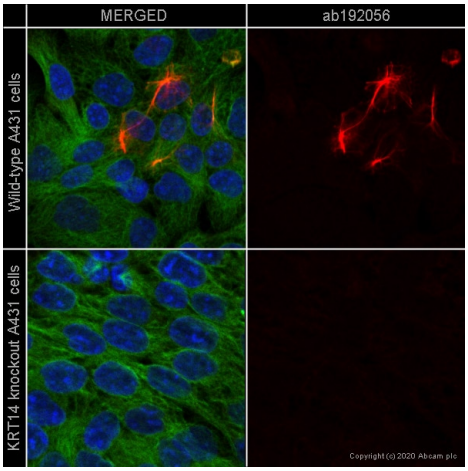
Lanes 1 - 4: Merged signal (red and green). Green - **ab51054** observed at 49 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

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 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.



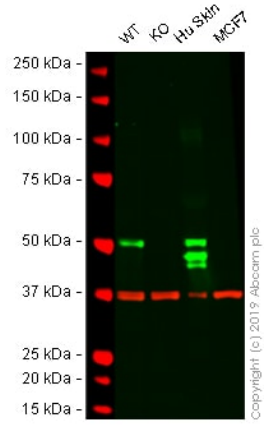
Immunocytochemistry/ Immunofluorescence - Human KRT14 (Cytokeratin 14) knockout A-431 cell line (ab261897)

ab181595 staining Cytokeratin 14 in wild-type A431 cells (top panel) and KRT14 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 **ab181595** 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).



Immunocytochemistry/ Immunofluorescence - Human KRT14 (Cytokeratin 14) knockout A-431 cell line (ab261897)

ab192056 staining KRT14 in wild-type A-431 cells (top panel) and KRT14 knockout A-431 cells (ab261897) (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 **ab192056** at 1/100 dilution and **ab195887** (Mouse monoclonal to alpha Tubulin - Alexa Fluor® 488) at 1/250 dilution 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 KRT14 (Cytokeratin 14) knockout A-431 cell line (ab261897)

All lanes : Anti-Cytokeratin 14 antibody [EPR17336] (**ab197893**) at 1/50000 dilution

Lane 1 : Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 2 : KRT14 knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 3 : Human skin whole tissue lysate
Lane 4 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

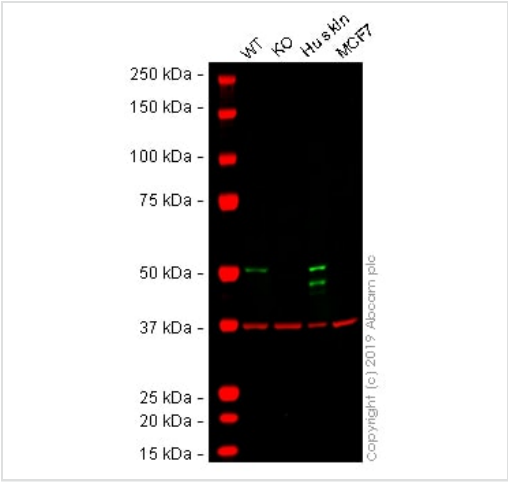
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 52 kDa
Observed band size: 52 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab197893** observed at 52 kDa. Red - loading control, **ab8245** (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 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.



Western blot - Human KRT14 (Cytokeratin 14) knockout A-431 cell line (ab261897)

All lanes : Anti-Cytokeratin 14 antibody [SP53] (**ab119695**) at 1/93 dilution

Lane 1 : Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 2 : KRT14 knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 3 : Human skin whole tissue lysate
Lane 4 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

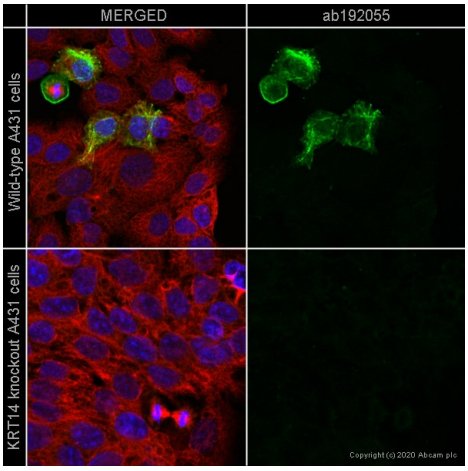
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 52 kDa
Observed band size: 52 kDa

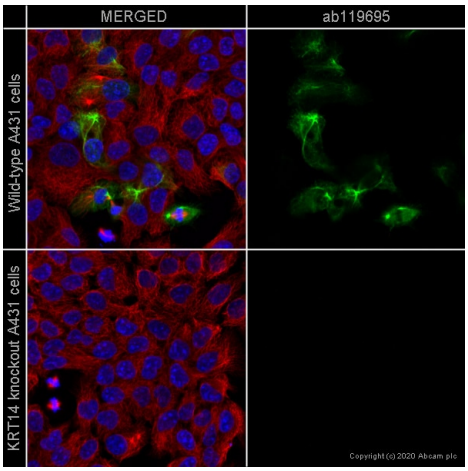
Lanes 1 - 4: Merged signal (red and green). Green - **ab119695** observed at 52 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

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.



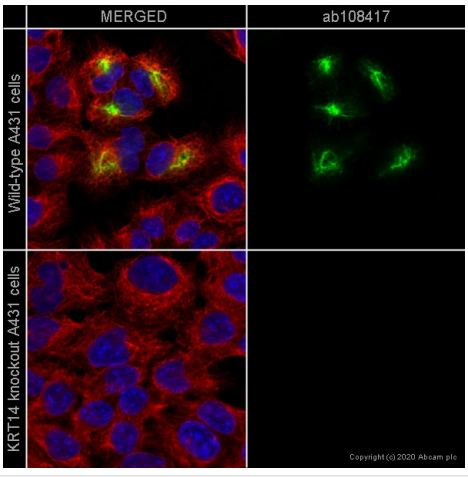
Immunocytochemistry/ Immunofluorescence - Human KRT14 (Cytokeratin 14) knockout A-431 cell line (ab261897)

ab192055 staining KRT14 in wild-type A-431 cells (top panel) and KRT14 knockout A-431 cells (ab261897) (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 **ab192055** at 1/100 dilution and **ab190573** (Rabbit monoclonal to alpha Tubulin - Alexa Fluor® 647) at 1/250 dilution overnight at 4°C. Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



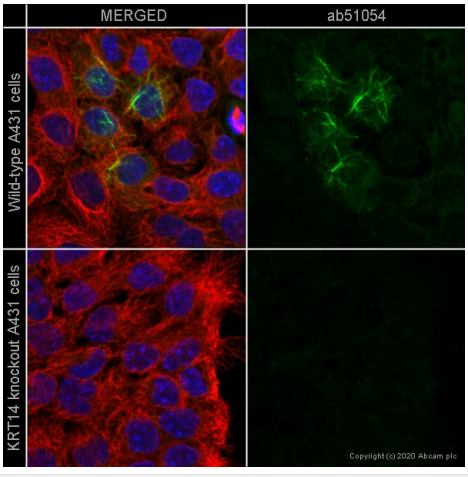
Immunocytochemistry/ Immunofluorescence - Human KRT14 (Cytokeratin 14) knockout A-431 cell line (ab261897)

ab119695 staining KRT14 in wild-type A-431 cells (top panel) and KRT14 knockout A-431 cells (ab261897) (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 **ab119695** at 5?g/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 ?g/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 ?g/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



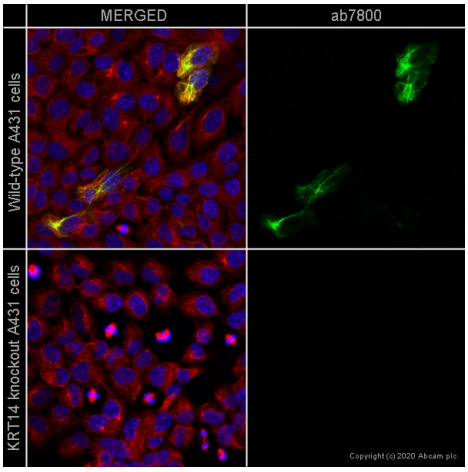
Immunocytochemistry/ Immunofluorescence - Human KRT14 (Cytokeratin 14) knockout A-431 cell line (ab261897)

ab108417 staining KRT14 in wild-type A-431 cells (top panel) and KRT14 knockout A-431 cells (ab261897) (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 **ab108417** at 1/100 dilution and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4^{o}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 ?g/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 ?g/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Immunocytochemistry/ Immunofluorescence - Human KRT14 (Cytokeratin 14) knockout A-431 cell line (ab261897)

ab51054 staining KRT14 in wild-type A-431 cells (top panel) and KRT14 knockout A-431 cells (ab261897) (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 **ab51054** at 1/100 dilution and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4^{o}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 ug/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 ug/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Immunocytochemistry/ Immunofluorescence - Human KRT14 (Cytokeratin 14) knockout A-431 cell line (ab261897)

ab7800 staining KRT14 in wild-type A-431 cells (top panel) and KRT14 knockout A-431 cells (ab261897) (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 **ab7800** at 1ug/ml concentration and **ab6046** (Rabbit polyclonal to beta Tubulin) at 1/1000 dilution overnight at 4^{o}C followed by a further incubation at room temperature for 1h with a goat secondary antibody to mouse IgG (Alexa Fluor® 488) (**ab150117**) at 2 ug/ml (shown in green) and a goat secondary antibody to rabbit IgG (Alexa Fluor® 594) (**ab150080**) at 2 ug/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).

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