abcam

Product datasheet

Human KRT8 (Cytokeratin 8) knockout HeLa cell line ab255400

5 Images

Overview

Product name	Human KRT8 (Cytokeratin 8) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 2 and 2 bp deletion in exon 2 and 4 bp deletion in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**Recommended control:** Human wild-type HeLa cell line (**ab255448**). 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, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and 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	Cervix
Cell type	epithelial
Disease	Adenocarcinoma
Gender	Female
STR Analysis	Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8, 12 CSF1PO: 9, 10
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	Together with KRT19, helps to link the contractile apparatus to dystrophin at the costameres of striated muscle.
Tissue specificity	Observed in muscle fibers accumulating in the costameres of myoplasm at the sarcolemma membrane in structures that contain dystrophin and spectrin. Expressed in gingival mucosa and hard palate of the oral cavity.
Involvement in disease	Cirrhosis
Sequence similarities	Belongs to the intermediate filament family.
Post-translational modifications	Phosphorylation on serine residues is enhanced during EGF stimulation and mitosis. Ser-74 phosphorylation plays an important role in keratin filament reorganization. O-glycosylated. O-GlcNAcylation at multiple sites increases solubility, and decreases stability by inducing proteasomal degradation. O-glycosylated (O-GlcNAcylated), in a cell cycle-dependent manner.
Cellular localization	Cytoplasm. Nucleus, nucleoplasm. Nucleus matrix.

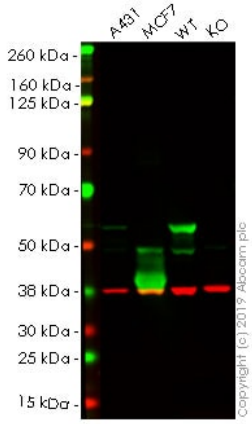
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab255400 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: 53 kDa.

Images



Western blot - Human KRT8 (Cytokeratin 8) knockout HeLa cell line (ab255400)

All lanes : Anti-Cytokeratin 8 antibody [M20] - Cytoskeleton Marker (**ab9023**) at 1/1000 dilution

Lane 1 : A431 cell lysate
Lane 2 : MCF7 cell lysate
Lane 3 : Wild-type HeLa cell lysate
Lane 4 : KRT8 knockout HeLa cell lysate

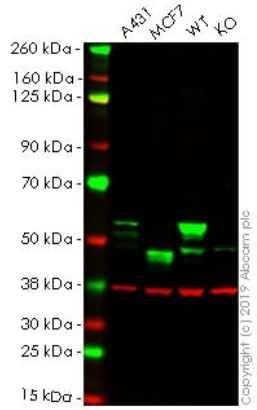
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) at 1/20000 dilution

Predicted band size: 53 kDa
Additional bands at: 37 kDa (possible Loading Control)

Lanes 1 - 4: Merged signal (red and green). Green - **ab9023** observed at 55 kDa. Red - loading control, **ab181602** observed at 37 kDa.

ab9023 was shown to react with Cytokeratin 8 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab255400 (knockout cell lysate **ab263785**) was used. Wild-type and Cytokeratin 8 knockout samples were subjected to SDS-PAGE. **ab9023** and Anti-GAPDH antibody EPR16891] - Loading Control (**ab181602**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human KRT8 (Cytokeratin 8) knockout HeLa cell line (ab255400)

All lanes : Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (**ab53280**) at 1/10000 dilution

Lane 1 : A431 cell lysate
Lane 2 : MCF7 cell lysate
Lane 3 : Wild-type HeLa cell lysate
Lane 4 : KRT8 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 53 kDa
Additional bands at: 37 kDa (possible Loading Control)

Lanes 1 - 4: Merged signal (red and green). Green - **ab53280** observed at 55 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab53280 was shown to react with Cytokeratin 8 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab255400 (knockout cell lysate **ab263785**) was used. Wild-type and Cytokeratin 8 knockout samples were subjected to SDS-PAGE. **ab53280** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 10000 (For unpurified use at 1/25,000 - 1/50,000) dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Sanger Sequencing - Human KRT8 knockout HeLa cell line (ab255400)

Allele-1: 4 bp deletion in exon 2.

Sanger Sequencing - Human KRT8 knockout HeLa cell line (ab255400)

Allele-2: 2 bp deletion in exon 2.

Sanger Sequencing - Human KRT8 knockout HeLa cell line (ab255400)

Allele-3: 1 bp insertion in exon 2.

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