abcam

Product datasheet

Human CANX (Calnexin) knockout HEK293T cell line ab255368

3 Images

Overview

Product name	Human CANX (Calnexin) knockout HEK293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 19 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 HEK293T cell line (ab255449). 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 -80ºC. Storage at -80ºC may result in loss of viability. 1. Thaw the vial in 37ºC water bath 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 (Click here to view haemocytometer protocol) 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. This should allow for confluency within 48 hours. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37ºC 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 for confluency (80-90% confluence) within 48 hours.
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.
Click here to view the Mammalian cell tissue culture protocol

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Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Viability	~90%
Adherent /Suspension	Adherent
Tissue	Kidney
Cell type	epithelial
STR Analysis	Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% DMSO, 2% Cellulose, methyl ether

Target

Function	Calcium-binding protein that interacts with newly synthesized glycoproteins in the endoplasmic reticulum. It may act in assisting protein assembly and/or in the retention within the ER of unassembled protein subunits. It seems to play a major role in the quality control apparatus of the ER by the retention of incorrectly folded proteins.
Sequence similarities	Belongs to the calreticulin family.
Cellular localization	Endoplasmic reticulum membrane. Melanosome. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

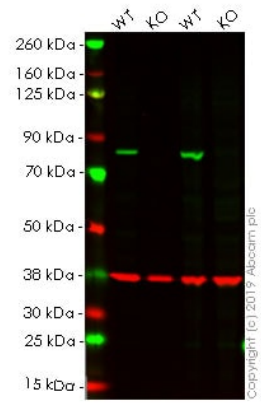
Applications

Our Abpromise guarantee covers the use of **ab255368** 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: 68 kDa.

Images



Western blot - Human CANX knockout HEK293T cell line (ab255368)

All lanes : Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker (ab133615) at 1/1000 dilution

Lane 1 : Wild-type Hap1 cell lysate
Lane 2 : CANX knockout Hap1 cell lysate
Lane 3 : Wild-type HEK-293T cell lysate
Lane 4 : CANX knockout HEK-293T cell lysate

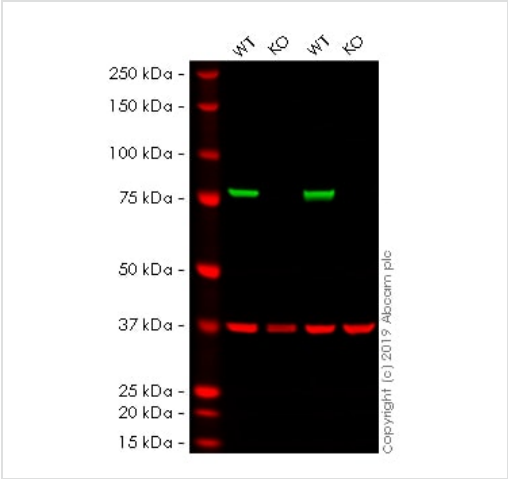
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 68 kDa
Additional bands at: 37 kDa (possible Loading Control)

Lanes 1 - 4: Merged signal (red and green). Green - ab133615 observed at 80 kDa. Red - loading control, ab8245 observed at 37 kDa.

ab133615 was shown to react with Calnexin in wild-type HEK-293T. Loss of signal was observed when knockout cell line ab255368 (knockout cell lysate ab263805) was used. Wild-type and Calnexin knockout samples were subjected to SDS-PAGE. ab133615 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 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.



Western blot - Human CANX knockout HEK293T cell line (ab255368)

All lanes : Anti-Calnexin antibody [EPR3632] (ab92573) at 1/20000 dilution

Lane 1 : Wild-type Hap1 cell lysate
Lane 2 : CANX knockout Hap1 cell lysate
Lane 3 : Wild-type HEK-293T cell lysate
Lane 4 : CANX knockout HEK-293T cell lysate

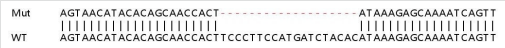
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 68 kDa
Observed band size: 37 kDa
why is the actual band size different from the predicted?

Lanes 1 - 4: Merged signal (red and green). Green - ab92573 observed at 80 kDa. Red - loading control, ab8245 observed at 37 kDa.

ab92573 was shown to react with Calnexin in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab255368 (knockout cell lysate ab263805) was used. Wild-type and Calnexin knockout samples were subjected to SDS-PAGE. ab92573 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 20000 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 CANX knockout HEK293T cell line (ab255368)

Homozygous: 19 bp deletion in exon2

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