abcam

Product datasheet

Human CAPN2 (Calpain 2) knockout HEK293T cell line ab266628

6 Images

Overview

Product name	Human CAPN2 (Calpain 2) knockout HEK293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 17 bp deletion in exon 4 and 1 bp insertion in exon 4 and 47 bp deletion in exon 4 and 8 bp deletion in exon 4
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
Antibiotic resistance	Puromycin 1.00µg/ml
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% DMSO, 2% Cellulose, methyl ether
Purity	Immunogen affinity purified

Target

Function	Calcium-regulated non-lysosomal thiol-protease which catalyze limited proteolysis of substrates involved in cytoskeletal remodeling and signal transduction.
Tissue specificity	Ubiquitous.
Sequence similarities	Belongs to the peptidase C2 family. Contains 1 calpain catalytic domain. Contains 3 EF-hand domains.
Cellular localization	Cytoplasm. Cell membrane. Translocates to the plasma membrane upon Ca(2+) binding.

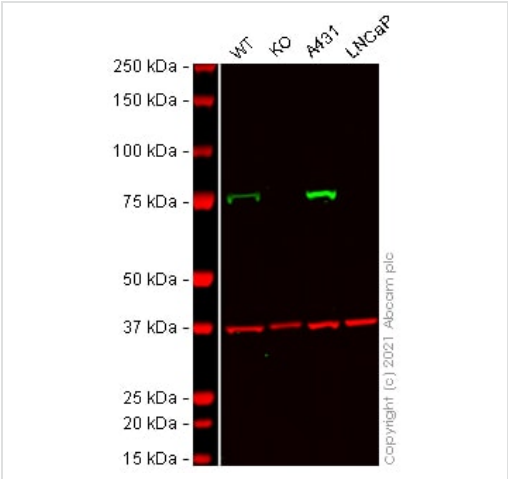
Applications

Our Abpromise guarantee covers the use of **ab266628** 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: 80 kDa.

Images



Western blot - Human CAPN2 (Calpain 2) knockout HEK293T cell line (ab266628)

All lanes : Anti-Calpain 2 antibody [EPR2562Y] (ab75994) at 1/2000 dilution

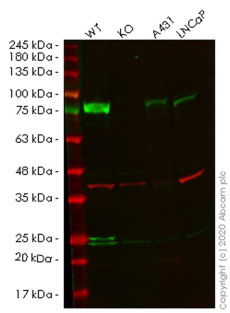
Lane 1 : Wild-type HEK-293T cell lysate at 40 µg
Lane 2 : CAPN2 knockout HEK-293T cell lysate at 40 µg
Lane 3 : A431 cell lysate at 20 µg
Lane 4 : LNCaP cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 80 kDa
Observed band size: 75 kDa
why is the actual band size different from the predicted?

Lanes 1 - 4: Merged signal (red and green). Green - ab75994 observed at 75 kDa. Red - loading control ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab75994 was shown to react with Calpain 2 in wild-type HEK-293T cells in Western blot with loss of signal observed in CAPN2 knockout cell line ab266628 (CAPN2 knockout cell lysate ab257379). Wild-type HEK-293T and CAPN2 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab75994 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 2000 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 h at room temperature before imaging.



Western blot - Human CAPN2 knockout HEK293T cell line (ab266628)

All lanes : Anti-Calpain 2 antibody [EPR5977] (ab126600) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : CAPN2 knockout HEK293T cell lysate
Lane 3 : A431 cell lysate
Lane 4 : LNCaP cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

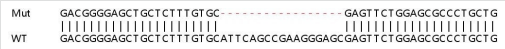
Predicted band size: 80 kDa
Observed band size: 80 kDa

Lanes 1-4: Merged signal (red and green). Green - ab126600 observed at 80 kDa. Red - loading control ab8245 observed at 36 kDa.

ab126600 Anti-Calpain 2 antibody [EPR5977] was shown to specifically react with Calpain 2 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266628 (knockout cell lysate ab257379) was used. Wild-type and Calpain 2 knockout samples were subjected to SDS-PAGE. ab126600 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at room temperature for 2. 5 hours 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.

Sanger Sequencing - Human CAPN2 knockout HEK293T cell line (ab266628)

Allele-1: 47 bp deletion in exon 4



Sanger Sequencing - Human CAPN2 knockout

HEK293T cell line (ab266628)

Allele-2: 17 bp deletion in exon 4.

Sanger Sequencing - Human CAPN2 knockout

HEK293T cell line (ab266628)

Allele-3: 8 bp deletion in exon 4.

Sanger Sequencing - Human CAPN2 knockout

HEK293T cell line (ab266628)

Allele-4: 1 bp insertion in exon 4.

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