

Product datasheet

Human CAPN2 (Calpain 2) knockout HEK-293T cell line ab266628

[6 Images](#)

Overview

Product name	Human CAPN2 (Calpain 2) knockout HEK-293T 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	<p>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.</p> <p>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: DMEM (High Glucose) + 10% FBS</p> <p>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.</p> <ol style="list-style-type: none">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 2×10^4 cells/cm². 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% CO₂. Cultures should be monitored daily. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods.</p>

A guide seeding density of 2×10^4 cells/cm² 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.

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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	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% Dimethylsulfoxide, 2% Cellulose, methyl ether

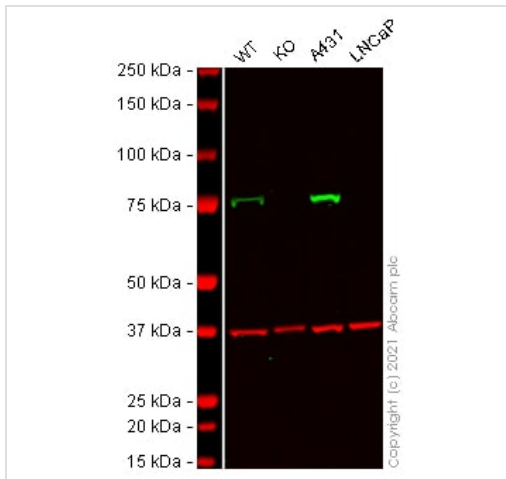
Target

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

The Abpromise guarantee 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.



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

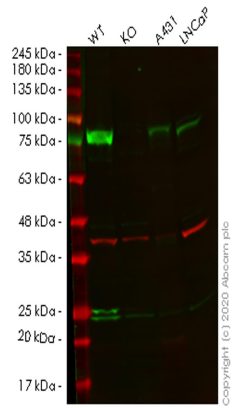
Performed under reducing conditions.

Predicted band size: 80 kDa

Observed band size: 75 kDa

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.

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Mut  GACGGGGAGCTGCTCTTTGTGC-----
      |||||
WT   GACGGGGAGCTGCTCTTTGTGCATTCAGCCGAAGGGAGCGAGTTCTGGAGCGCCCTGCTG
  
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Allele-1: 47 bp deletion in exon 4

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

Mut GACGGGGAGCTGCTCTTTGTGC-----GAGTTCGGAGCGCCCTGCTG
 |||
 WT GACGGGGAGCTGCTCTTTGTGCATTCAGCCGAAGGGAGCGAGTTCGGAGCGCCCTGCTG

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

Allele-2: 17 bp deletion in exon 4.

Mut GACGGGGAGCTGCTCTTTGTGC-----GAAGGGAGCGAGTTCGGAGCGCCCTGCTG
 |||
 WT GACGGGGAGCTGCTCTTTGTGCATTCAGCCGAAGGGAGCGAGTTCGGAGCGCCCTGCTG

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

Allele-3: 8 bp deletion in exon 4.

Mut GACGGGGAGCTGCTCTTTGTGCTATTCAGCCGAAGGGAGCGAGTTCGGAGCGCCCTGCT
 |||
 WT GACGGGGAGCTGCTCTTTGTGC ATTCAGCCGAAGGGAGCGAGTTCGGAGCGCCCTGCT

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

Allele-4: 1 bp insertion in exon 4.

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