abcam

Product datasheet

Human ACE2 knockout Caco-2 cell line ab273731

1 References 5 Images

Overview

Product name	Human ACE2 knockout Caco-2 cell line
Parental Cell Line	Caco 2
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 62 bp deletion in exon 2
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	1
General notes	**Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** EMEM + 20% 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 $1x10^4$ 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 $1x10^4$ 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 (approx $8x10^4$-$1x10^5$ cells/cm^2). 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.

Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Colon
Cell type	epithelial
Disease	Adenocarcinoma
Gender	Male
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	Carboxypeptidase which converts angiotensin I to angiotensin 1-9, a peptide of unknown function, and angiotensin II to angiotensin 1-7, a vasodilator. Also able to hydrolyze apelin-13 and dynorphin-13 with high efficiency. May be an important regulator of heart function. In case of human coronaviruses SARS and HCoV-NL63 infections, serve as functional receptor for the spike glycoprotein of both coronaviruses.
Tissue specificity	Expressed in endothelial cells from small and large arteries, and in arterial smooth muscle cells. Expressed in lung alveolar epithelial cells, enterocytes of the small intestine, Leydig cells and Sertoli cells (at protein level). Expressed in heart, kidney, testis, and gastrointestinal system.
Sequence similarities	Belongs to the peptidase M2 family.
Post-translational modifications	N-glycosylation on Asn-90 may limit SARS infectivity.
Cellular localization	Secreted and Cell membrane.

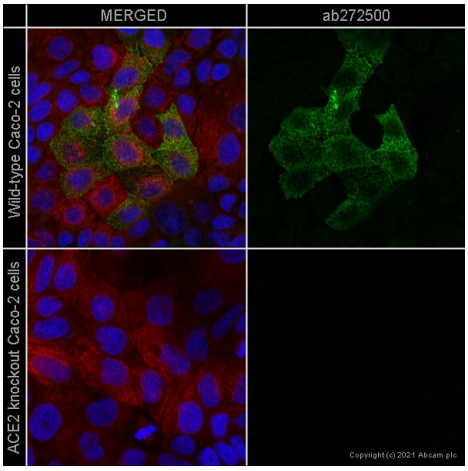
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab273731 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

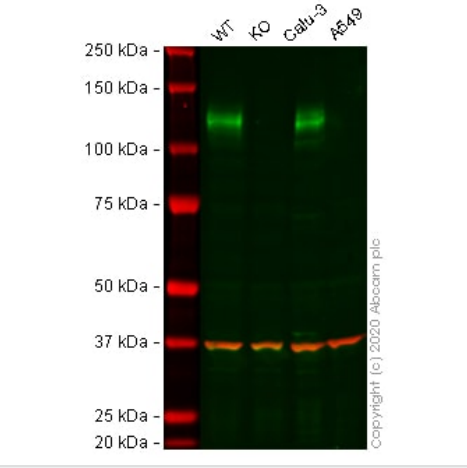
Application	Abreviews	Notes
WB		Use at an assay dependent concentration.

Images



Immunocytochemistry - Human ACE2 knockout Caco-2 cell line (ab273731)

ab272500 staining ACE2 in wild-type Caco2 cells (top panel) and ACE2 knockout Caco2 cells (ab273731) (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Tween 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 **ab272500** at 10µg/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).



Western blot - Human ACE2 knockout Caco 2 cell line (ab273731)

All lanes : Anti-ACE2 antibody [EPR4435(2)] (**ab108252**) at 1/1000 dilution

Lane 1 : Wild-type Caco-2 cell lysate
Lane 2 : ACE2 knockout Caco-2 cell lysate
Lane 3 : Calu-3 cell lysate
Lane 4 : A549 cell lysate

Lysates/proteins at 30 µg per lane.

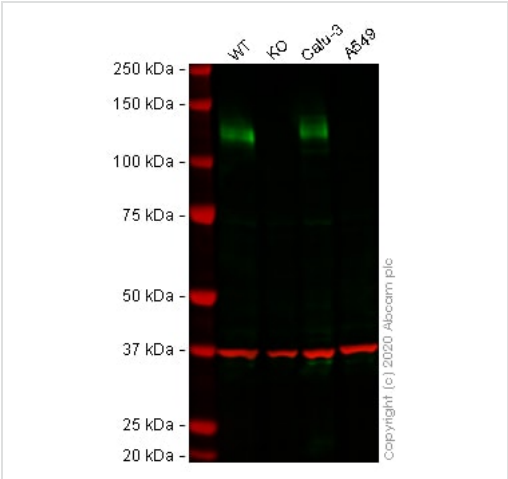
Performed under reducing conditions.

Observed band size: 125 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab108252** observed at 125 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab108252 was shown to react with ACE2 in Caco-2 wild-type cells in western blot with loss of signal observed in ACE2 knockout cell line ab273731 (knockout cell lysate **ab275516**). Wild-type and ACE2 knockout Caco-2 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab108252** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 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.



Western blot - Human ACE2 knockout Caco 2 cell line (ab273731)

All lanes : Anti-ACE2 antibody [EPR4436] (**ab108209**) at 1/1000 dilution

Lane 1 : Wild-type Caco-2 cell lysate
Lane 2 : ACE2 knockout Caco-2 cell lysate
Lane 3 : Calu-3 cell lysate
Lane 4 : A549 cell lysate

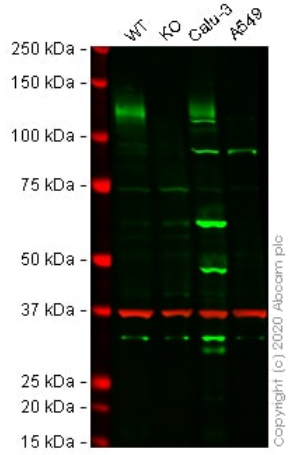
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Observed band size: 125 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab108209** observed at 125 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab108209 was shown to react with ACE2 in Caco-2 wild-type cells in western blot with loss of signal observed in ACE2 knockout cell line ab273731 (knockout cell lysate **ab275516**). Wild-type and ACE2 knockout Caco-2 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab108209** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 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.



Western blot - Human ACE2 knockout Caco 2 cell line (ab273731)

All lanes : Anti-ACE2 antibody (**ab65863**) at 1 µg/ml

Lane 1 : Wild-type Caco-2 cell lysate
Lane 2 : ACE2 knockout Caco-2 cell lysate
Lane 3 : Calu-3 cell lysate
Lane 4 : A549 cell lysate

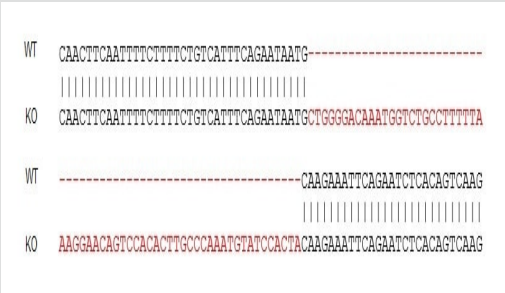
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Observed band size: 125 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab65863** observed at 125 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab65863 was shown to react with ACE2 in Caco-2 wild-type cells in western blot with loss of signal observed in ACE2 knockout cell line ab273731 (knockout cell lysate **ab275516**). Wild-type and ACE2 knockout Caco-2 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab65863** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at 1 µg/ml 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.



Sanger Sequencing - Human ACE2 knockout Caco 2 cell line (ab273731)

Homozygous: 62 bp deletion in exon 2

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