abcam

Product datasheet

Human CD276 knockout HEK-293T cell line ab266658

8 图像

概述

产品名称	人CD276 knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: Insertion of the selection cassette in exon 2
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Sanger Sequencing, Western Blot (WB)
经测试应用	**适用于:** WB, ICC, Flow Cyt
Biosafety level	2
常规说明	**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 -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.

性能

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Kidney
Cell type	epithelial
Gender	Female
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
存放说明	Shipped on Dry Ice. Store in liquid nitrogen.
存储溶液	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

靶标

功能	May participate in the regulation of T-cell-mediated immune response. May play a protective role in tumor cells by inhibiting natural-killer mediated cell lysis as well as a role of marker for detection of neuroblastoma cells. May be involved in the development of acute and chronic transplant rejection and in the regulation of lymphocytic activity at mucosal surfaces. Could also play a key role in providing the placenta and fetus with a suitable immunological environment throughout pregnancy. Both isoform 1 and isoform 2 appear to be redundant in their ability to modulate CD4 T-cell responses. Isoform 2 is shown to enhance the induction of cytotoxic T-cells and selectively stimulates interferon gamma production in the presence of T-cell receptor signaling.
组织特异性	Ubiquitous but not detectable in peripheral blood lymphocytes or granulocytes. Weakly expressed in resting monocytes. Expressed in dendritic cells derived from monocytes. Expressed in epithelial cells of sinonasal tissue. Expressed in extravillous trophoblast cells and Hofbauer cells of the first trimester placenta and term placenta.
序列相似性	Belongs to the immunoglobulin superfamily. BTN/MOG family. Contains 2 Ig-like C2-type (immunoglobulin-like) domains. Contains 2 Ig-like V-type (immunoglobulin-like) domains.
细胞定位	Membrane.

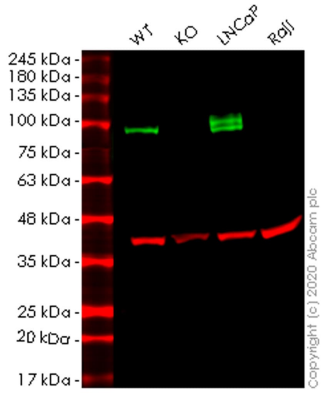
应用

The Abpromise guarantee	**Abpromise™承诺保证使用**ab266658**于以下的**经测试应用

“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		Use at an assay dependent concentration. Predicted molecular weight: 57 kDa.
ICC		Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration.

图片



Western blot - Human CD276 knockout HEK293T cell line (ab266658)

All lanes : Anti-CD276 antibody [SP206] (**ab227670**) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : CD276 knockout HEK293T cell lysate
Lane 3 : LNCaP cell lysate
Lane 4 : Raji cell lysate

Lysates/proteins at 20 µg per lane.

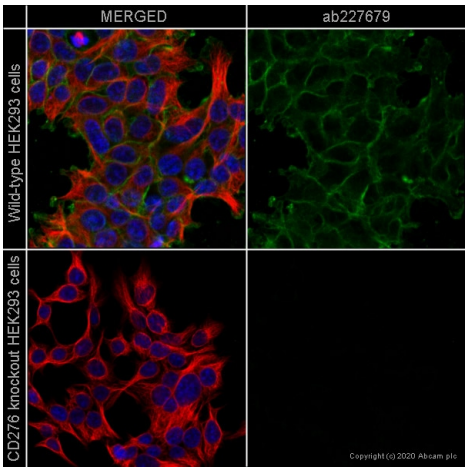
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 57 kDa
Observed band size: 90-110 kDa

Lanes 1-4: Merged signal (red and green). Green - **ab227670** observed at 90-110 kDa. Red - loading control **ab8245** observed at 36 kDa.

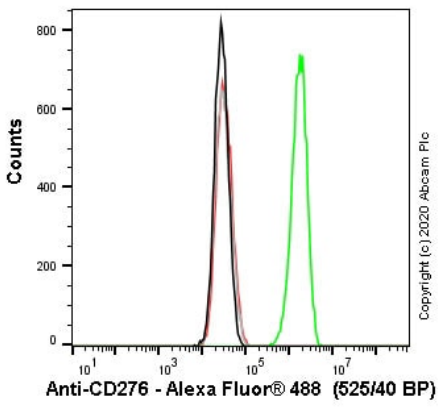
ab227670 Anti-CD276 antibody [SP206] was shown to specifically react with CD276 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266658 (knockout cell lysate **ab257097**) was used. Wild-type and CD276 knockout samples were subjected to SDS-PAGE. **ab227670** and Anti-GAPDH 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.



Immunocytochemistry/ Immunofluorescence - Human CD276 knockout HEK-293T cell line (ab266658)

ab227679 staining CD276 in wild-type HEK293 cells (top panel) and CD276 knockout HEK293 cells (ab266658) (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 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 **ab227679** at 1/100 dilution 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).



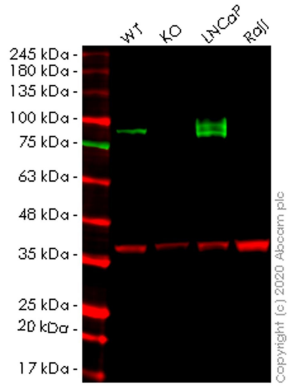
Flow Cytometry - Human CD276 knockout HEK293T cell line (ab266658)

Flow cytometry overlay histogram showing wild-type HEK293 (green line) and CD276 knockout HEK293 cells (ab266658) stained with **ab134161** (red line). The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab134161**) ($1x10^6$ in 100µl at 0.2 µg/ml) for 30 min at 4°C.

The secondary antibody Goat anti-rabbit IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150081**) was used at 1/2000 for 30 min at 4°C.

Isotype control antibody was Rabbit IgG (monoclonal) (**ab172730**) used at the same concentration and conditions as the primary antibody (wild-type HEK293 - black line CD276 HEK293 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Western blot - Human CD276 knockout HEK293T cell line (ab266658)

All lanes : Anti-CD276 antibody [EPR20115] (**ab219648**) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : CD276 knockout HEK293T cell lysate
Lane 3 : LNCaP cell lysate
Lane 4 : Raji cell lysate

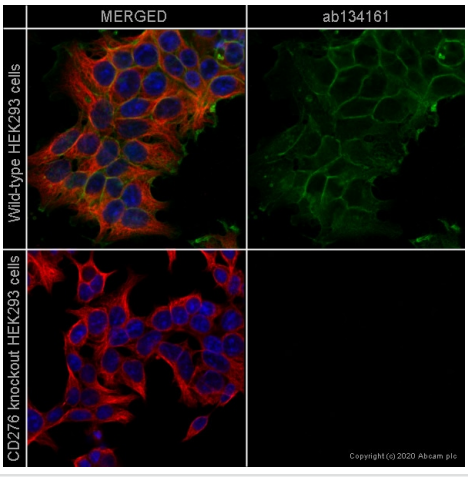
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 57 kDa
Observed band size: 90-110 kDa

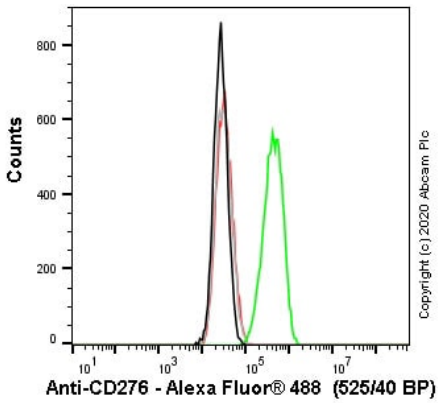
Lanes 1-4: Merged signal (red and green). Green - **ab219648** observed at 90-110 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab219648 Anti-CD276 antibody [EPR20115] was shown to specifically react with CD276 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266658 (knockout cell lysate **ab257097**) was used. Wild-type and CD276 knockout samples were subjected to SDS-PAGE. **ab219648** 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.



Immunocytochemistry/ Immunofluorescence - Human CD276 knockout HEK-293T cell line (ab266658)

ab134161 staining CD276 in wild-type HEK293 cells (top panel) and CD276 knockout HEK293 cells (ab266658) (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 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 **ab134161** at 1µg/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).



Flow Cytometry - Human CD276 knockout HEK293T cell line (ab266658)

Flow cytometry overlay histogram showing wild-type HEK293 (green line) and CD276 knockout HEK293 cells (ab266658) stained with **ab89133** (red line). The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab89133**) ($1x10^6$ in 100µl at 0.2 µg/ml) for 30 min at 4°C.

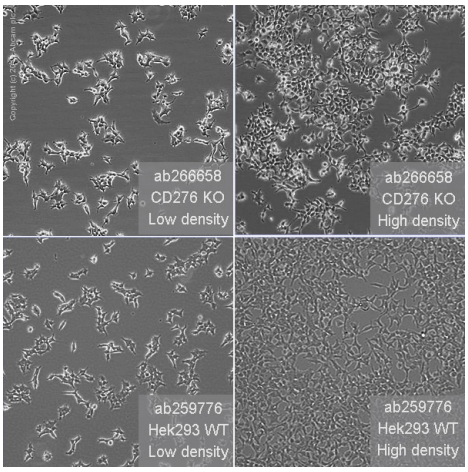
The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150117**) was used at 1/2000 for 30 min at 4°C.

Isotype control antibody was mouse IgG1κ (**ab170190**) used at the same concentration and conditions as the primary antibody (wild-type HEK293 - black line CD276 HEK293 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

Sanger Sequencing - Human CD276 knockout HEK293T cell line (ab266658)

Homozygous: Insertion of the selection cassette in exon 2



Cell Culture - Human CD276 knockout HEK293T cell line (ab266658)

Representative images of CD276 knockout HEK293T cells, low and high confluency examples (top left and right respectively) and wild-type HEK293T cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using a EVOS XL Core microscope.

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