abcam

Product datasheet

Anti-CD276 antibody [EPNCIR122] ab134161

敲除验证 重组 RabMAb

16 References 12 图像

概述

产品名称	Anti-CD276抗体[EPNCIR122]
描述	兔单克隆抗体[EPNCIR122] to CD276
宿主	Rabbit
经测试应用	**适用于:** IHC-Fr, WB, IP, Flow Cyt, ICC/IF **不适用于:** IHC-P
种属反应性	**与反应:** Mouse, Human
免疫原	Recombinant full length protein corresponding to Human CD276. Alternate injections with Human recombinant protein and Human CD276 over-expressing CHO cells. Database link: **Q5ZPR3** (Peptide available as **ab182198**)
阳性对照	WB: LnCaP, CHO-K1 cells lysates transfected with human and mouse CD276. Flow Cyt: HEK293 and THP1 cells. ICC/IF: HEK293 cells.
常规说明	This antibody was developed as part of a collaboration between Epitomics, the National Cancer Institute's Center for Cancer Research and the lab of Brad St. Croix. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.

存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPNCIR122
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab134161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
IHC-Fr		Use at an assay dependent concentration.
WB		1/200. Predicted molecular weight: 57 kDa.
IP		1/10 - 1/100.
Flow Cyt		1/1000 - 1/10000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		Use a concentration of 1 µg/ml.

应用说明 Is unsuitable for IHC-P.

靶标

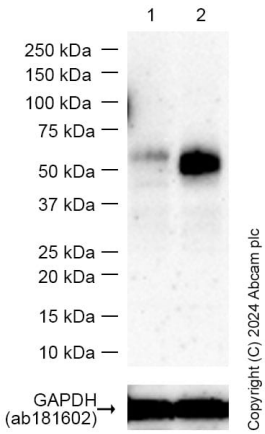
功能 May participate in the regulation of T-cell-mediated immune response. May play a protective role in tumor cells by inhibiting natural-killer mediated cell lysis as well as a role of marker for detection of neuroblastoma cells. May be involved in the development of acute and chronic transplant rejection 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.

图片



Western blot - Anti-CD276 antibody [EPNCIR122] (ab134161)

All lanes : Anti-CD276 antibody [EPNCIR122] (ab134161) at 1/1000 dilution

Lane 1 : LLC (Mouse Lewis lung carcinoma cell) whole cell lysate (boiled)
Lane 2 : LLC (Mouse Lewis lung carcinoma cell) whole cell lysate (unboiled)

Lysates/proteins at 20 µg per lane.

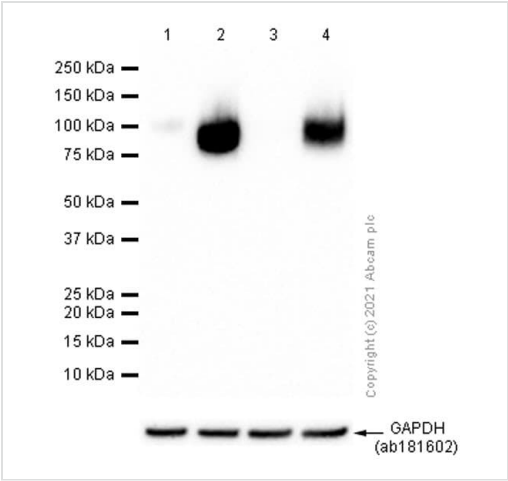
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 57 kDa
Observed band size: 45-66 kDa

Exposure time: 180 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

We recommend not to boil the samples after lysis to get desired WB results.
Mouse CD276 (B7-H3) has a molecular weight about 45–66 kDa, depending on the glycosylation levels [PMID: 34366684].



Western blot - Anti-CD276 antibody [EPNCIR122] (ab134161)

All lanes : Anti-CD276 antibody [EPNCIR122] (ab134161) at 1/1000 dilution

Lane 1 : HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate boiled. with NFDM/TBST
Lane 2 : HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate unboiled. with NFDM/TBST
Lane 3 : LNCaP (Human prostate carcinoma epithelial cell) whole cell lysate boiled. with NFDM/TBST
Lane 4 : LNCaP (Human prostate carcinoma epithelial cell) whole cell lysate unboiled. with NFDM/TBST

Lysates/proteins at 20 µg per lane.

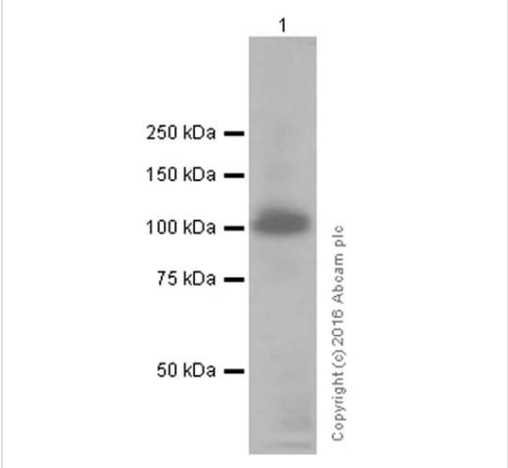
Blocking peptides at 5 % per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution (Goats anti-rabbit IgG (H+L) Peroxidase Conjugated)

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

We recommend not to boil the samples after lysis to get desired WB results.

CD276 (B7-H3) has a molecular weight of about 90–100 kDa, depending on the glycosylation levels. [PMID:22473715].



Western blot - Anti-CD276 antibody [EPNCIR122] (ab134161)

Anti-CD276 antibody [EPNCIR122] (ab134161) at 1/200 dilution + CHO (hamster ovary epithelial cell) whole cell lysates transfected with human CD276 at 15 µg

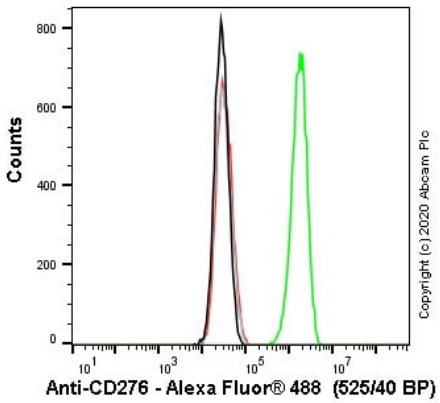
Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution

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

Exposure time: 3 minutes

Blocking/dilution buffer: 5% NFDM/TBST

Performed using purified antibody.



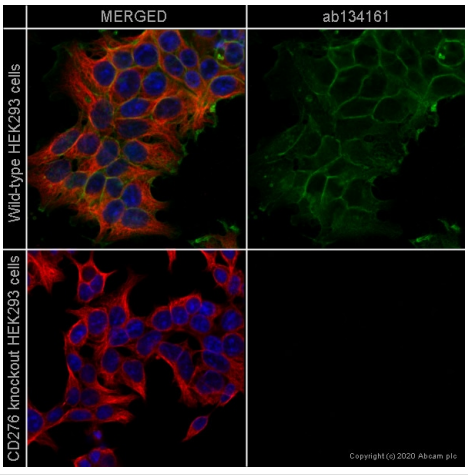
Flow Cytometry - Anti-CD276 antibody [EPNCIR122] (ab134161)

Flow cytometry overlay histogram showing wild-type HEK293 (green line) and CD276 knockout HEK293 (**ab266658**) cells 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.

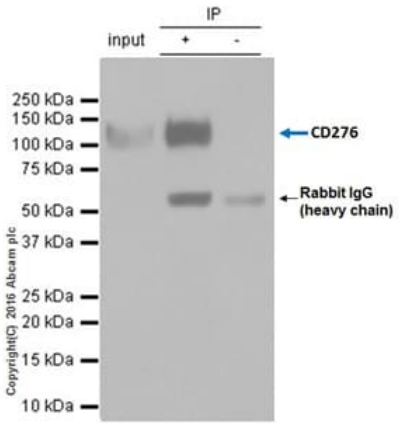
Isotype control antibody was Rabbit IgG (monoclonal) (**ab172730**) used at the same concentration and conditions as the primary antibody (wild-type HEK293 - black line CD276 HEK293 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

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



Immunocytochemistry/ Immunofluorescence - Anti-CD276 antibody [EPNCIR122] (ab134161)

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).



Immunoprecipitation - Anti-CD276 antibody [EPNCIR122] (ab134161)

CD276 was immunoprecipitated from 10 ug of HEK 293 (human embryonic kidney epithelial cell) whole cell lysate with ab134161 at 1/40 diltuion. Western blot was permformed from the immunoprecipitate using ab 134161 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/500 dilution.

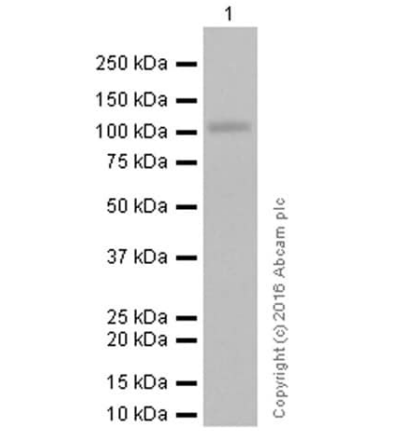
Lane 1: HEK 293 whole cell lysate 10 ug (Input).

Lane 2: ab134161 IP in HEK 293 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab134161 in HEK 293 whole cell lysate.

Blocking/dilution buffer: 5% NFDM/TBST

Performed using purified antibody.



Western blot - Anti-CD276 antibody [EPNCIR122] (ab134161)

Anti-CD276 antibody [EPNCIR122] (ab134161) at 1/1000 dilution + LNCap (human prostate carcinoma epithelial cell) whole cell lysate at 15 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

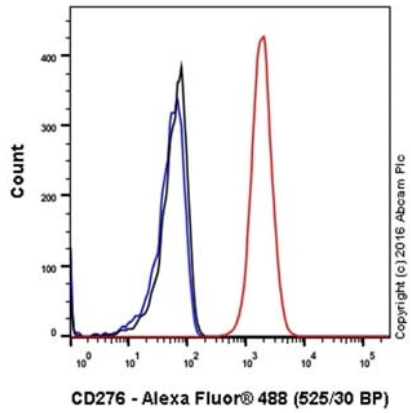
Predicted band size: 57 kDa

Observed band size: 110 kDa

Exposure time: 3 minutes

Blocking/dilution buffer: 5% NFDM/TBST

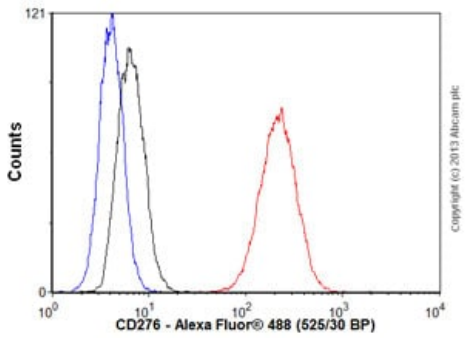
Performed using purified antibody.



Flow Cytometry - Anti-CD276 antibody [EPNCIR122] (ab134161)

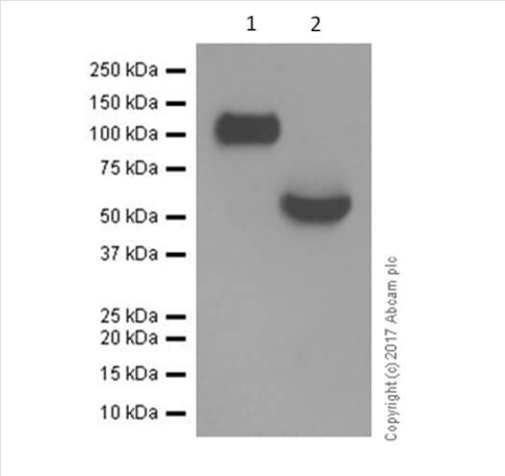
Overlay histogram showing THP-1 (human monocytic leukemia monocyte) cells stained with ab134161 (red line). The cells were fixed with 4% paraformaldehyde and then permeabilized with 90% methanol. The cells were incubated with the antibody (ab134161) at 1/80 dilution. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150077**) at 1/2000 dilution. Isotype control antibody (black line) was rabbit monoclonal IgG (**ab172730**) used under the same conditions. Unlabelled sample (blue line) was also used as a control.

Performed using purified antibody.



Flow Cytometry - Anti-CD276 antibody [EPNCIR122] (ab134161)

Overlay histogram showing THP1 cells stained with ab134161 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% human serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab134161, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Western blot - Anti-CD276 antibody [EPNCIR122] (ab134161)

All lanes : Anti-CD276 antibody [EPNCIR122] (ab134161) at 0.264 µg/ml

Lane 1 : CHO-K1 cell lysates, transfected with Human CD276
Lane 2 : CHO-K1 cell lysates, transfected with Mouse CD276.

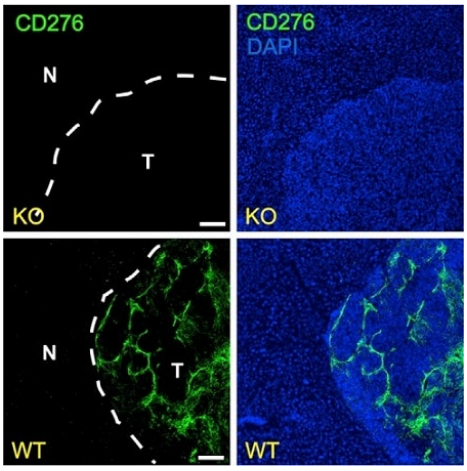
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 57 kDa

Exposure time: 3 minutes

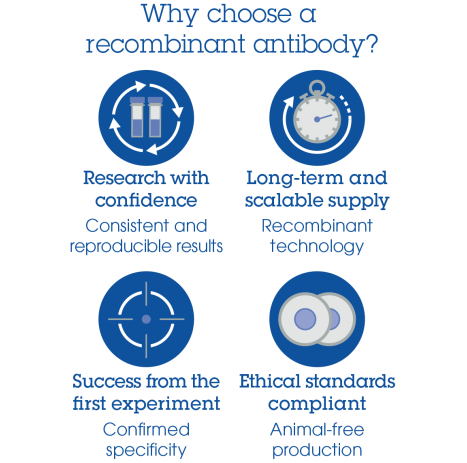
Blocking/dilution buffer: 5% NFDM/TBST



Immunohistochemistry of mouse MC38 colon liver metastasis. Staining CD276 with ab134161 (green). Normal tissue (N)/tumor tissue (T) margins are indicated by a white dash.

Immunohistochemistry (Frozen sections) - Anti-CD276 antibody [EPNCIR122] (ab134161)

Image from Seaman S et al. Cancer Cell, 31, 501-515. Fig2.D doi:10.1016/j.ccell.2017.03.005. with permission from Elsevier



Anti-CD276 antibody [EPNCIR122] (ab134161)

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