abcam

ab134161

Anti-B7-H3/CD276 antibody [EPNCIR122]

Anti-B7-H3/CD276 antibody [EPNCIR122] (ab134161) is a rabbit monoclonal antibody detecting B7-H3/CD276 in **Western Blot, Flow Cytometry, IP, IHC-Fr, ICC/IF**. Suitable for **Human, Mouse**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 20 publications

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPNCIR122
Purification technique	Affinity purification Protein A
Concentration	1.854 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/10 - 1/100
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	1/1000 - 1/10000
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Human
Dilution info	1/200
Notes	-

Expected

Species	Mouse

Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Not recommended

Species	Mouse, Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1 µg/mL
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-Fr

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.

Notes	-

Target data

See full target information B7-H3/CD276

Function	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.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Anti-CD276 antibody [EPNCIR122] (ab134161) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt, ICC/IF, IHC-Fr, IP, WB in human, mouse samples.

Anti-CD276 antibody [EPNCIR122] (ab134161) has been cited over 20 times in peer reviewed journals and is trusted by the scientific community.

Abcam's high quality manufacturing and validation processes ensure Anti-CD276 antibody [EPNCIR122] (ab134161) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

The specificity of Anti-CD276 antibody [EPNCIR122] (ab134161) has been confirmed by testing in knockout samples.

Anti-CD276 antibody [EPNCIR122] (ab134161) specifically detects CD276 (UniProt ID: Q5ZPR3; Molecular weight: 54kDa) and is sold in 100 µL and 1 mL selling sizes.

Conjugation-ready, carrier free format available for antibody clone EPNCIR122 - ab256585.

Antibody clone EPNCIR122 is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 488, Alexa Fluor® 594, Alexa Fluor® 555 (ab218280, ab218282, ab218283).

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.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Species reactivity
Rat: We have preliminary internal testing data to indicate this antibody may not react with this species.
Please contact us for more information.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	CD276 also known as B7-H3 is an immunoregulatory protein with a mass of approximately 57 kDa although post-translational modifications can increase its observed size. It is a member of the B7 family which is part of the immunoglobulin superfamily. CD276 is widely expressed in various tissues including placenta liver heart and also in many tumor cells. It is observed in lesser amounts in normal tissues making it an interesting target for cancer research. Various assays like HEK293 and HEK293T cell confluency studies often use CD276 to explore its expression under different conditions and seeding densities which are represented in confluency charts.
Biological function summary	CD276 modulates immune responses by acting as a co-inhibitory ligand impacting T-cell proliferation and cytokine synthesis. Not part of a known complex it plays a role in the adaptive immune system by providing negative feedback regulation that can inhibit the immune response. This capability has implications in preventing autoimmunity but may also contribute to tumor immune evasion by reducing effective anti-tumor responses. The B7-H3/CD276 interaction with receptors such as IL receptor family members suggests its diverse immunomodulatory functions.

Pathways	CD276 participates in the T-cell receptor (TCR) signaling pathways influencing immune checkpoint pathways. It interacts with other immune checkpoint proteins like PD-L1 and CTLA-4 but its exact receptor partner remains unidentified. The presence of CD276 in these pathways highlights its role in controlling T-cell activation and tolerance. By modulating TCR signals and impacting downstream effects CD276 serves as a regulatory point that can balance immune activation and inhibition critical in immune-mediated conditions.
Associated diseases and disorders	CD276 has strong associations with cancer particularly in solid tumors such as prostate and breast cancer. It is often overexpressed in malignant tissues contributing to immune evasion by tumors. Researchers propose it as a potential target for cancer immunotherapy due to this attribute. CD276's link to disorders like asthma has also been suggested where its interaction with proteins involved in inflammatory pathways such as IL response elements can exacerbate the condition. This implicates it as a dual-role protein in both disease progression and potential therapeutic targeting.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

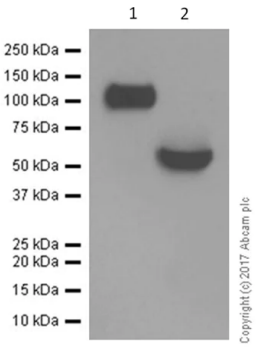
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

11 product images



Western blot - Anti-B7-H3/CD276 antibody [EPNCIR122] (ab134161)

B7-H3/CD276 Western blot staining using rabbit Anti-B7-H3/CD276 antibody

Blocking/dilution buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-B7-H3/CD276 antibody [EPNCIR122] (ab134161) at 0.264 µg/mL

Lane 1:
CHO-K1 cell lysates, transfected with Human CD276 at 20 µg

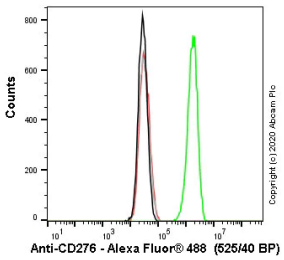
Lane 2:
CHO-K1 cell lysates, transfected with Mouse CD276. at 20 µg

Secondary

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

Predicted band size: 57 kDa

Exposure time: 3min



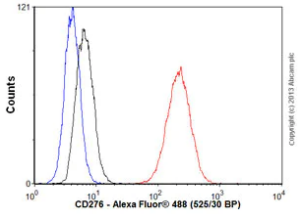
Flow Cytometry - Anti-B7-H3/CD276 antibody [EPNCIR122] (ab134161)

B7-H3/CD276 Flow Cytometry staining using rabbit Anti-B7-H3/CD276 antibody

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.

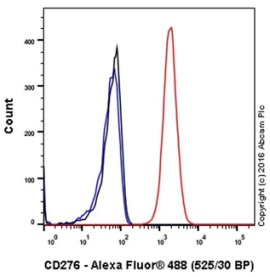


Flow Cytometry - Anti-B7-H3/CD276 antibody [EPNCIR122] (ab134161)

B7-H3/CD276 Flow Cytometry staining using rabbit Anti-B7-H3/CD276 antibody

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

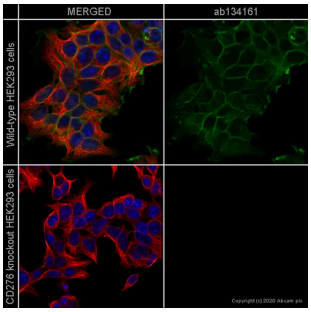
as a control. Acquisition of >5000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Flow Cytometry - Anti-B7-H3/CD276 antibody [EPNCIR122] (ab134161)

B7-H3/CD276 Flow Cytometry staining using rabbit Anti-B7-H3/CD276 antibody

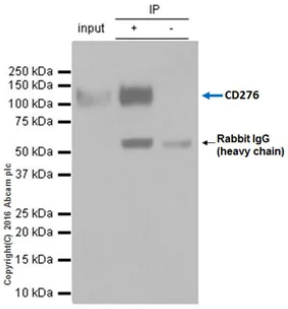
Overlay histogram showing THP-1 (human monocytic leukemia monocyte) cells stained with ab134161 (red line). The cells were fixed with 4% paraformaldehyde and then permeabilized with 90% methanol. The cells were incubated with the antibody (ab134161) at 1/80 dilution. The secondary antibody used was Alexa 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.



Immunocytochemistry/ Immunofluorescence - Anti-B7-H3/CD276 antibody [EPNCIR122] (ab134161)

B7-H3/CD276 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-B7-H3/CD276 antibody

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-B7-H3/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.

All lanes:
Immunoprecipitation - Anti-B7-H3/CD276 antibody [EPNCIR122] (ab134161)

Predicted band size: 57 kDa

Exposure time: 3s

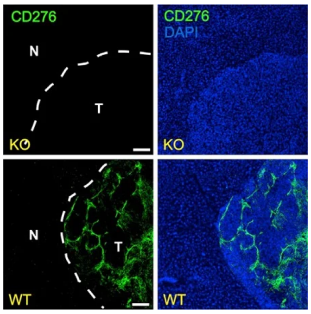
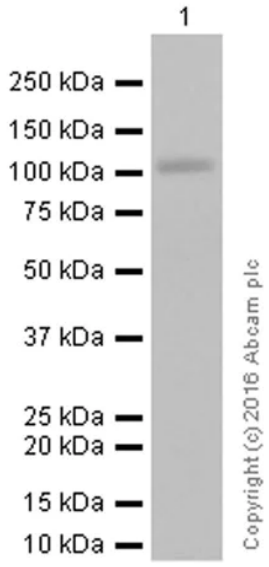


Image from Seaman S et al. Cancer Cell, 31, 501-515. Fig2.D doi:10.1016/j.ccell.2017.03.005. with permission from Elsevier

Immunohistochemistry (Frozen sections) - Anti-B7-H3/CD276 antibody [EPNCIR122] (ab134161)

B7-H3/CD276 Immunohistochemistry (Frozen sections) staining using rabbit Anti-B7-H3/CD276 antibody

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.



Western blot - Anti-B7-H3/CD276 antibody [EPNCIR122] (ab134161)

B7-H3/CD276 Western blot staining of LNCap (human prostate carcinoma epithelial cell) whole cell lysate using rabbit Anti-B7-H3/CD276 antibody

Blocking/dilution buffer: 5% NFDM/TBST
Performed using purified antibody.

All lanes:
Western blot - Anti-B7-H3/CD276 antibody [EPNCIR122] (ab134161) at 1/1000 dilution

All lanes:
LNCap (human prostate carcinoma epithelial cell) whole cell lysate at 15 µg

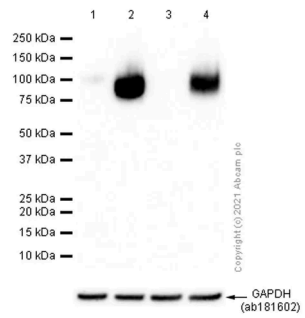
Secondary

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

Predicted band size: 112 kDa, 388 kDa, 47 kDa, 55 kDa, 57 kDa, 72 kDa, 83 kDa, 97 kDa

Observed band size: 100 kDa, 110 kDa, 140 kDa, 388 kDa, 50-70 kDa

Exposure time: 3min



Western blot - Anti-B7-H3/CD276 antibody [EPNCIR122] (ab134161)

B7-H3/CD276 Western blot staining using rabbit Anti-B7-H3/CD276 antibody

We recommend not to boil the samples after lysis to get desired WB results.
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

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

All lanes:
Western blot - Anti-B7-H3/CD276 antibody [EPNCIR122] (ab134161) at 1/1000 dilution

Lane 1:
HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate boiled. at 20 µg

Lane 2:
HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate unboiled. at 20 µg

Lane 3:
LNCaP (Human prostate carcinoma epithelial cell) whole cell lysate boiled. at 20 µg

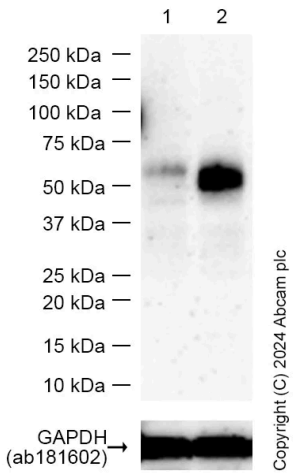
Lane 4:
LNCaP (Human prostate carcinoma epithelial cell) whole cell lysate unboiled. at 20 µg

Secondary

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

Predicted band size: 57 kDa

Observed band size: 90 kDa



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Western blot - Anti-B7-H3/CD276 antibody [EPNCIR122] (ab134161)

B7-H3/CD276 Western blot staining using rabbit Anti-B7-H3/CD276 antibody

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].

All lanes:
Western blot - Anti-B7-H3/CD276 antibody [EPNCIR122] (ab134161) at 1/1000 dilution

Lane 1:
LLC (Mouse Lewis lung carcinoma cell) whole cell lysate (boiled) at 20 µg

Lane 2:
LLC (Mouse Lewis lung carcinoma cell) whole cell lysate (unboiled) at 20 µg

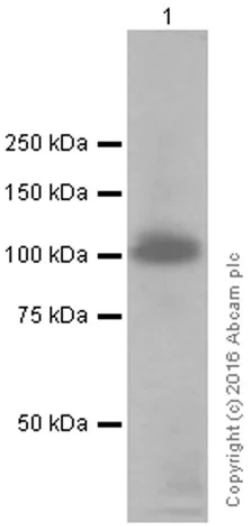
Secondary

All lanes:
Western blot - 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: 180s



Western blot - Anti-B7-H3/CD276 antibody [EPNCIR122] (ab134161)

B7-H3/CD276 Western blot staining of CHO (hamster ovary epithelial cell) whole cell lysates transfected with human CD276 using rabbit Anti-B7-H3/CD276 antibody

Blocking/dilution buffer: 5% NFDM/TBST
Performed using purified antibody.

All lanes:
Western blot - Anti-B7-H3/CD276 antibody [EPNCIR122] (ab134161) at 1/200 dilution

All lanes:
CHO (hamster ovary epithelial cell) whole cell lysates transfected with human CD276 at 15 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/10000 dilution

Predicted band size: 57 kDa

Observed band size: 110 kDa

Exposure time: 3min

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.