BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

Anti-CD276 Antibody Picoband®

Catalog Number: A02220-3

About CD276

Cluster of Differentiation 276 (CD276) or B7 Homolog 3 (B7-H3) is a human protein encoded by the CD276 gene. The protein encoded by this gene belongs to the immunoglobulin superfamily, and thought to participate in the regulation of T-cell-mediated immune response. Studies show that while the transcript of this gene is ubiquitously expressed in normal tissues and solid tumors, the protein is preferentially expressed only in tumor tissues. Additionally, it was observed that the 3' UTR of this transcript contains a target site for miR29 microRNA, and there is an inverse correlation between the expression of this protein and miR29 levels, suggesting regulation of expression of this gene product by miR29. Alternatively spliced transcript variants encoding different isoforms have been found for this gene.

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Overview

Product Name	Anti-CD276 Antibody Picoband®
Reactive Species	Human
Description	Boster Bio Anti-CD276 Antibody Picoband® catalog # A02220-3. Tested in ELISA, Flow Cytometry, IHC, WB applications. This antibody reacts with Human. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	ELISA, Flow Cytometry, IHC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl, 0.2 mg Na2HPO4.
Storage Instructions	At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freezing and thawing.
Host	Rabbit
Uniprot ID	Q5ZPR3

Technical Details

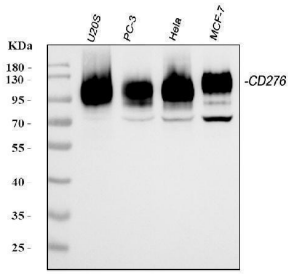
Immunogen	E.coli-derived human CD276 recombinant protein (Position: L29-Q458).

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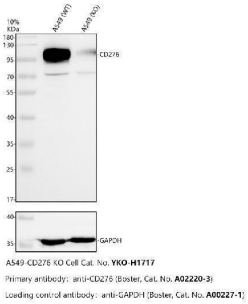
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P).
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 μg/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.25-0.5 ug/ml, Human Immunohistochemistry(Paraffin-embedded Section), 2-5 ug/ml, Human Flow Cytometry (Fixed), 1-3 ug/1x10^6 cells, Human ELISA, 0.1-0.5 ug/ml, - Note: For flow cytometry (FCM), suitable for fixed cells only. Not suitable for FACS or surface flow cytometry using live cells.

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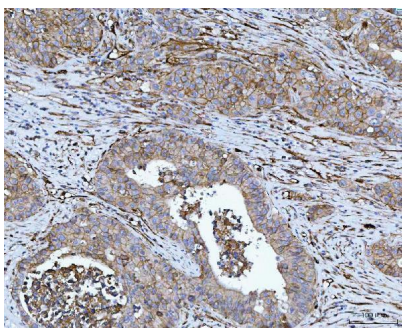
Anti-CD276 Antibody Picoband® (A02220-3) Images



Western blot analysis of CD276 using anti-CD276 antibody (A02220-3). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human U2OS whole cell lysates, Lane 2: human PC-3 whole cell lysates, Lane 3: human Hela whole cell lysates, Lane 4: human MCF-7 whole cell lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-CD276 antigen affinity purified polyclonal antibody (Catalog # A02220-3) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for CD276 at approximately 90-100 kDa. The expected band size for CD276 is at 100 kDa.



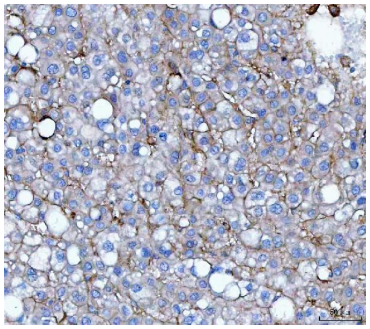
Western blot analysis of B7-H3/CD276 using anti-B7-H3/CD276 antibody (A02220-3). Electrophoresis was performed on a 10% SDS-PAGE gel at 80V (Stacking gel) / 120V (Resolving gel) for 2 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human A549- WT whole cell lysates, Lane 2: human A549-CD276 KO whole cell lysates.After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-B7-H3/CD276 antigen affinity purified polyclonal antibody (A02220-3) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an ECL Plus Western Blotting Substrate (Catalog # AR1196-200) with Tanon 5200 system. A specific band was detected for B7-H3/CD276 at approximately 90-100 kDa. The expected band size for B7-H3/CD276 is at 57 kDa.



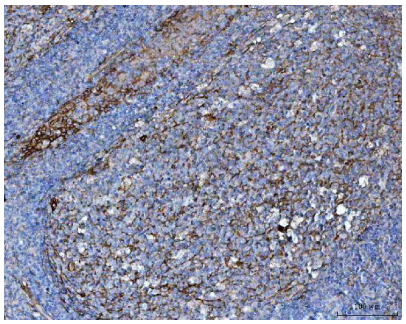
IHC analysis of CD276 using anti-CD276 antibody (A02220-3). CD276 was detected in a paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-CD276 Antibody (A02220-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using

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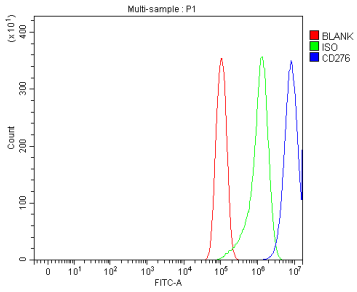
HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.



IHC analysis of CD276 using anti-CD276 antibody (A02220-3). CD276 was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-CD276 Antibody (A02220-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.



IHC analysis of CD276 using anti-CD276 antibody (A02220-3). CD276 was detected in a paraffin-embedded section of human tonsil tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-CD276 Antibody (A02220-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.



Flow Cytometry analysis of PC-3 cells using anti-CD276 antibody (A02220-3). Overlay histogram showing PC-3 cells stained with A02220-3 (Blue line). The cells were fixed with 4% paraformaldehyde and blocked with 10% normal goat serum. And then incubated with rabbit anti-CD276 Antibody (A02220-3, 1 ug/1x106 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x106 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x106) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

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