

Anti-CD43/SPN Antibody Picoband™ (monoclonal, 4I3)

Catalog # ABO14886

Product Information

Application	WB, IHC, FC
Primary Accession	P16150
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-CD43/SPN Antibody Picoband™ (monoclonal, 4I3) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500 µg/ml.

Additional Information

Gene ID	6693
Other Names	Leukosialin, GPL115, Galactoglycoprotein, GALGP, Leukocyte sialoglycoprotein, Sialophorin, CD43, CD43 cytoplasmic tail, CD43-ct, CD43ct, SPN, CD43
Calculated MW	40322
Application Details	Western blot, 0.1-0.5 µg/ml, Human Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml, Human, Mouse, Rat, By Heat Flow Cytometry, 1-3 µg/1x10 ⁶ cells, Human
Subcellular Localization	Membrane. Single-pass type I membrane protein. Microvillus. Uropodium. Nucleus. PML body.
Tissue Specificity	Cell surface of thymocytes, T-lymphocytes, neutrophils, plasma cells and myelomas.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg NaN ₃ .
Clone Names	Clone: 4I3
Immunogen	E.coli-derived human CD43 recombinant protein (Position: A272-P400). Human CD43 shares 72% and 73% amino acid (aa) sequence identity with mouse and rat CD43, respectively.

Cross Reactivity	No cross-reactivity with other proteins.
Storage	Store 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 freeze-thaw cycles.

Protein Information

Name	SPN
Synonyms	CD43
Function	Predominant cell surface sialoprotein of leukocytes which regulates multiple T-cell functions, including T-cell activation, proliferation, differentiation, trafficking and migration. Positively regulates T-cell trafficking to lymph-nodes via its association with ERM proteins (EZR, RDX and MSN) (By similarity). Negatively regulates Th2 cell differentiation and predisposes the differentiation of T-cells towards a Th1 lineage commitment. Promotes the expression of IFN-gamma by T-cells during T-cell receptor (TCR) activation of naive cells and induces the expression of IFN-gamma by CD4(+) T-cells and to a lesser extent by CD8(+) T-cells (PubMed: 18036228). Plays a role in preparing T-cells for cytokine sensing and differentiation into effector cells by inducing the expression of cytokine receptors IFNGR and IL4R, promoting IFNGR and IL4R signaling and by mediating the clustering of IFNGR with TCR (PubMed: 24328034). Acts as a major E-selectin ligand responsible for Th17 cell rolling on activated vasculature and recruitment during inflammation. Mediates Th17 cells, but not Th1 cells, adhesion to E- selectin. Acts as a T-cell counter-receptor for SIGLEC1 (By similarity).
Cellular Location	Membrane; Single-pass type I membrane protein. Cell projection, microvillus {ECO:0000250 UniProtKB:P13838}. Cell projection, uropodium {ECO:0000250 UniProtKB:P15702}. Note=Localizes to the uropodium and microvilli via its interaction with ERM proteins (EZR, RDX and MSN) {ECO:0000250 UniProtKB:P13838, ECO:0000250 UniProtKB:P15702}
Tissue Location	Cell surface of thymocytes, T-lymphocytes, neutrophils, plasma cells and myelomas

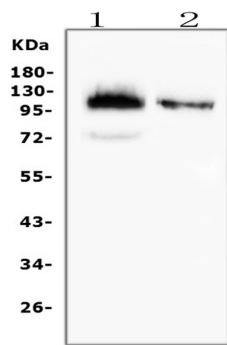
Background

CD43, also known as leukosialin or sialophorin, is a transmembrane cell surface protein that in humans is encoded by the SPN gene. It is mapped to 16p11.2. It is a major sialoglycoprotein on the surface of human T lymphocytes, monocytes, granulocytes, and some B lymphocytes, which is important for immune function and may be part of a physiologic ligand-receptor complex involved in T-cell activation. Expression of CD43 is deficient and/or defective in the X-chromosome-linked immunodeficiency disorder Wiscott-Aldrich syndrome, suggesting that CD43 have a role in T-cell activation. T-cell activation requires the removal of CD43 from the immunologic synapse to allow efficient engagement of the TCR with molecules on the APC.

Images

Figure 1. Western blot analysis of CD43 using anti-CD43 antibody (M01296-1).

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 50ug of sample under reducing conditions.



Lane 1: human K562 whole cell lysates

Lane 2: human HL-60 whole cell lysates

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-CD43 antigen affinity purified monoclonal antibody (Catalog # M01296-1) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for CD43 at approximately 115KD. The expected band size for CD43 is at 40KD.

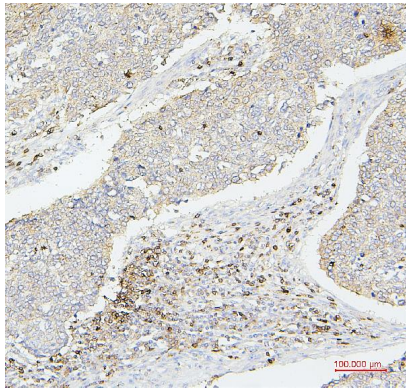


Figure 2. IHC analysis of CD43 using anti-CD43 antibody (M01296-1).

CD43 was detected in paraffin-embedded section of human lung cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-CD43 Antibody (M01296-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

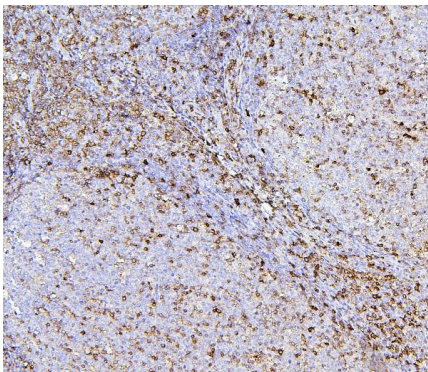


Figure 3. IHC analysis of CD43 using anti-CD43 antibody (M01296-1).

CD43 was detected in paraffin-embedded section of human tonsil tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-CD43 Antibody (M01296-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

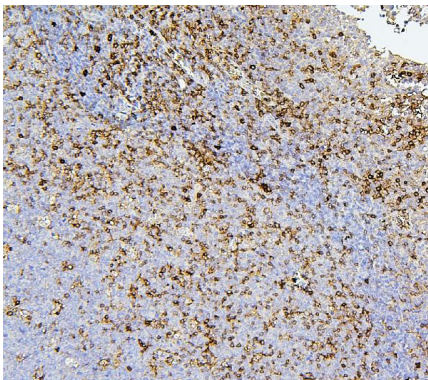


Figure 4. IHC analysis of CD43 using anti-CD43 antibody (M01296-1).

CD43 was detected in paraffin-embedded section of human tonsil tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-CD43 Antibody (M01296-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

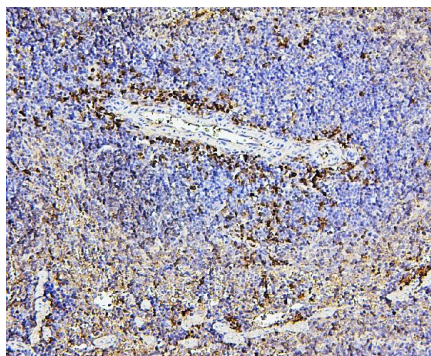


Figure 5. IHC analysis of CD43 using anti-CD43 antibody (M01296-1).
CD43 was detected in paraffin-embedded section of mouse spleen tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 $\mu\text{g}/\text{ml}$ mouse anti-CD43 Antibody (M01296-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

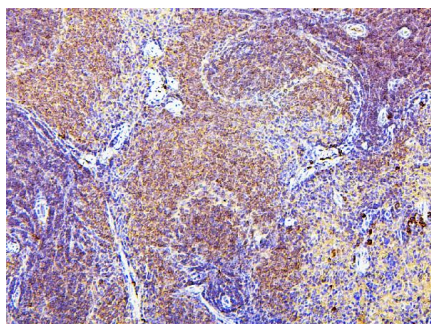


Figure 6. IHC analysis of CD43 using anti-CD43 antibody (M01296-1).
CD43 was detected in paraffin-embedded section of rat spleen tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 $\mu\text{g}/\text{ml}$ mouse anti-CD43 Antibody (M01296-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

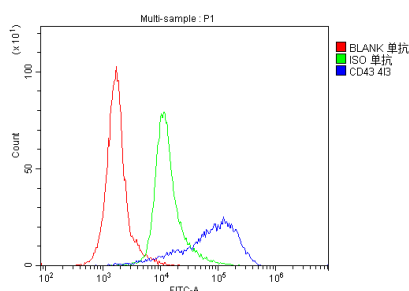


Figure 7. Flow Cytometry analysis of human PBMC cells using anti-CD43 antibody (M01296-1).
Overlay histogram showing human PBMC cells stained with M01296-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-CD43 Antibody (M01296-1, 1 $\mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 $\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 $\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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