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Anti-CD2AP Antibody Picoband™ (monoclonal, 5F8)

Catalog # ABO14802

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	Q9Y5K6
Host	Mouse
Isotype	Mouse IgG1
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-CD2AP Antibody Picoband™ (monoclonal, 5F8) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.

Additional Information

Gene ID	23607
Other Names	CD2-associated protein, Adapter protein CMS, Cas ligand with multiple SH3 domains, CD2AP
Calculated MW	71451
Application Details	Western blot, 0.1-0.5 □g/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml Immunocytochemistry/Immunofluorescence, 5 □g/ml Flow Cytometry, 1-3 □g/1x10^6 cells
Subcellular Localization	Cytoplasm, cytoskeleton. Cell projection, ruffle. Colocalizes with F-actin and BCAR1/p130Cas in membrane ruffles. Located at podocyte slit diaphragm between podocyte foot processes (By similarity). During late anaphase and telophase, concentrates in the vicinity of the midzone microtubules and in the midbody in late telophase.
Tissue Specificity	Widely expressed in fetal and adult tissues.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Clone Names	Clone: 5F8
Immunogen	E. coli-derived human CD2AP recombinant protein (Position: K253-K337).
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	CD2AP
Function	Seems to act as an adapter protein between membrane proteins and the actin cytoskeleton (PubMed:10339567). In collaboration with CBLC, modulates the rate of RET turnover and may act as regulatory checkpoint that limits the potency of GDNF on neuronal survival. Controls CBLC function, converting it from an inhibitor to a promoter of RET degradation (By similarity). May play a role in receptor clustering and cytoskeletal polarity in the junction between T-cell and antigen-presenting cell (By similarity). May anchor the podocyte slit diaphragm to the actin cytoskeleton in renal glomerolus. Also required for cytokinesis (PubMed:15800069). Plays a role in epithelial cell junctions formation (PubMed:22891260).
Cellular Location	Cytoplasm, cytoskeleton. Cell projection, ruffle. Cell junction. Note=Colocalizes with F-actin and BCAR1/p130Cas in membrane ruffles (PubMed:10339567). Located at podocyte slit diaphragm between podocyte foot processes (By similarity). During late anaphase and telophase, concentrates in the vicinity of the midzone microtubules and in the midbody in late telophase (PubMed:15800069). {ECO:0000250|UniProtKB:Q9JLQ0, ECO:0000269|PubMed:10339567, ECO:0000269|PubMed:15800069}
Tissue Location	Widely expressed in fetal and adult tissues.

Background

CD2-associated protein is a protein that in humans is encoded by the CD2AP gene. This gene encodes a scaffolding molecule that regulates the actin cytoskeleton. The protein directly interacts with filamentous actin and a variety of cell membrane proteins through multiple actin binding sites, SH3 domains, and a proline-rich region containing binding sites for SH3 domains. The cytoplasmic protein localizes to membrane ruffles, lipid rafts, and the leading edges of cells. It is implicated in dynamic actin remodeling and membrane trafficking that occurs during receptor endocytosis and cytokinesis. Haploinsufficiency of this gene is implicated in susceptibility to glomerular disease.

Images

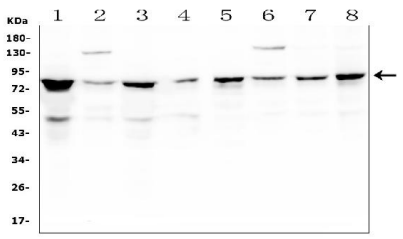


Figure 1. Western blot analysis of CD2AP using anti-CD2AP antibody (M01756).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human K562 whole cell lysate,
Lane 2: human A431 whole cell lysate,
Lane 3: human HEK293 whole cell lysate,
Lane 4: human U20S whole cell lysate,
Lane 5: human HL-60 whole cell lysate,
Lane 6: human MCF-7 whole cell lysate,
Lane 7: human Hela whole cell lysate,
Lane 8: human PANC-1 whole cell lysate,
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-CD2AP antigen affinity purified monoclonal antibody (Catalog # M01756) 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 CD2AP at approximately 80KD. The expected band size for CD2AP is at 71KD.

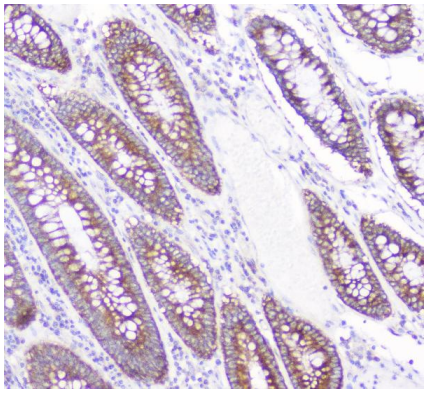


Figure 2. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).
CD2AP was detected in paraffin-embedded section of human colon cancer. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-CD2AP Antibody (M01756) 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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

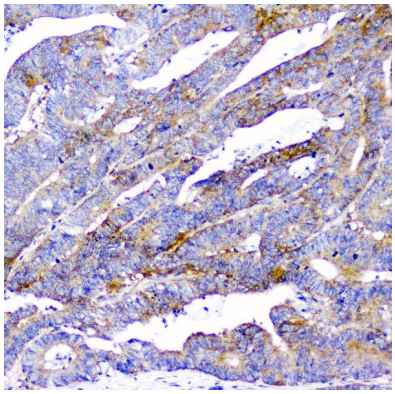


Figure 3. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).
CD2AP was detected in paraffin-embedded section of human colon cancer. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epi1ope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-CD2AP Antibody (M01756) 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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

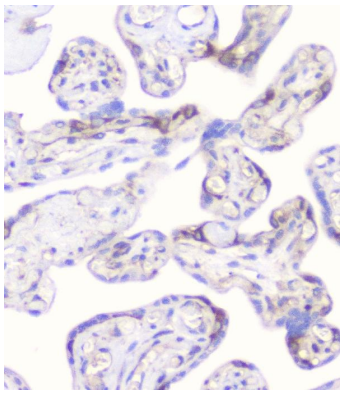
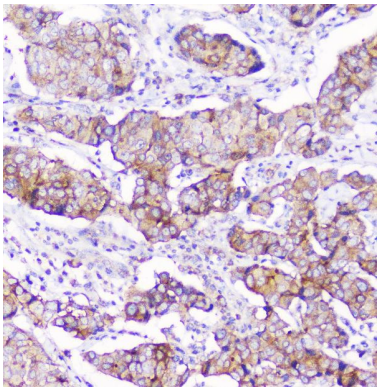


Figure 4. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).
CD2AP was detected in paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-CD2AP Antibody (M01756) 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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

Figure 5. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).
CD2AP was detected in paraffin-embedded section of



human mammary cancer. 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-CD2AP Antibody (M01756) 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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

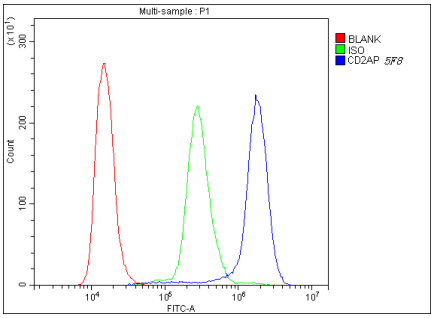


Figure 6. Flow Cytometry analysis of K562 cells using anti-D2AP antibody (M01756).
Overlay histogram showing K562 cells stained with M01756 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-D2AP Antibody (M01756,1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

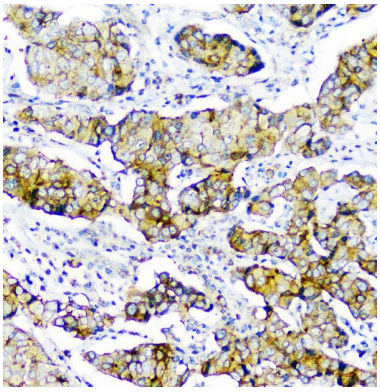


Figure 7. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).
CD2AP was detected in paraffin-embedded section of human mammary cancer. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-CD2AP Antibody (M01756) 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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

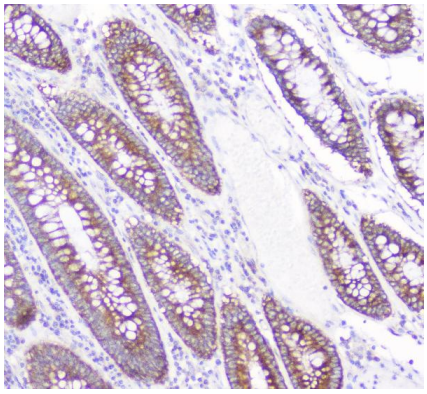
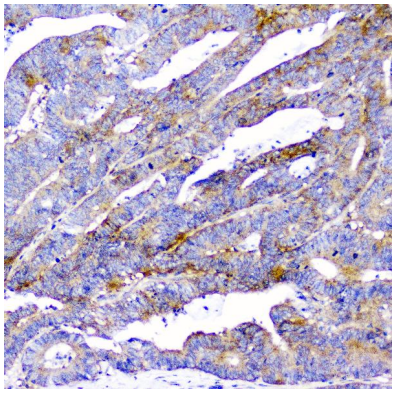


Figure 8. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).
CD2AP was detected in paraffin-embedded section of human colon cancer. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-CD2AP Antibody (M01756) 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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

Figure 9. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).
CD2AP was detected in paraffin-embedded section of human colon cancer. 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-CD2AP Antibody (M01756) 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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

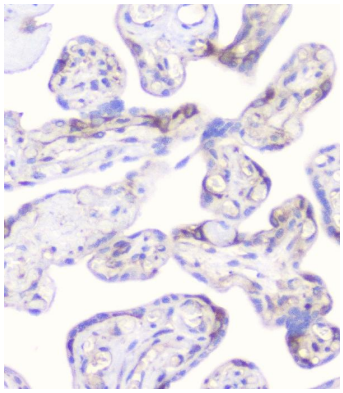


Figure 10. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).
CD2AP was detected in paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-CD2AP Antibody (M01756) 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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

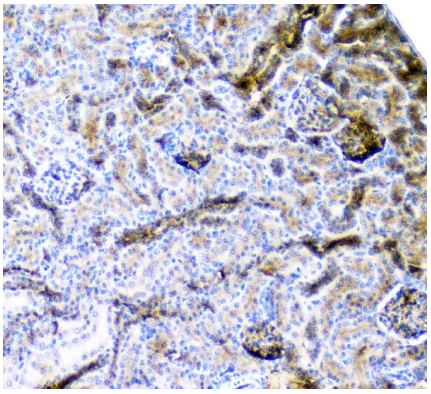


Figure 11. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).
CD2AP was detected in paraffin-embedded section of mouse kidney tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-CD2AP Antibody (M01756) 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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

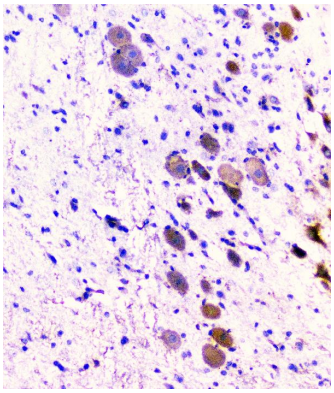
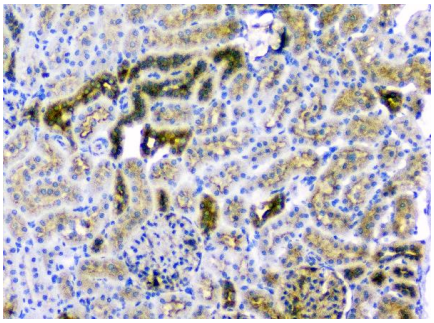


Figure 12. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).
CD2AP was detected in paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-CD2AP Antibody (M01756) 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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

Figure 13. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).
CD2AP was detected in paraffin-embedded section of rat kidney tissue. 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-CD2AP Antibody (M01756) 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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

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