

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

Catalog Number: M01756

About CD2AP

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.

Overview

Product Name	Anti-CD2AP Antibody Picoband™ (monoclonal, 5F8)
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-CD2AP Antibody Picoband™ (monoclonal, 5F8) catalog # M01756. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Monoclonal 5F8
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Storage Instructions	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.
Host	Mouse
Uniprot ID	Q9Y5K6

Technical Details

Immunogen	E. coli-derived human CD2AP recombinant protein (Position: K253-K337).
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Mouse IgG (EK1001) for Western blot, and HRP Conjugated anti-Mouse IgG Super Vision Assay Kit (SV0001-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Mouse IgG1
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.

Suggested Dilutions

Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.

If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.

Some PubMed article(s) citing the expression level of this target are as follows:

Boster Bio's internal QC testing used:

Western blot, 0.1-0.5ug/ml

Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml

Immunocytochemistry/Immunofluorescence, 5 ug/ml

Flow Cytometry, 1-3ug/1x10⁶ cells

Anti-CD2AP Antibody Picoband™ (monoclonal, 5F8) (M01756) Images

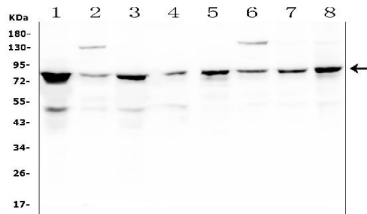


Figure 1. Western blot analysis of CD2AP using anti-CD2AP antibody (M01756).

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 U2OS 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 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-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.

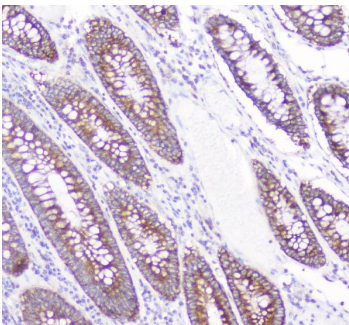


Figure 2. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).

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 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

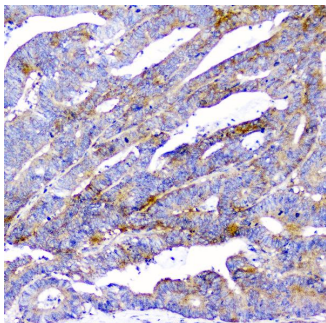


Figure 3. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).

CD2AP was detected in paraffin-embedded section of human colon cancer. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/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 Streptavidin-Biotin-Complex

(SABC)(Catalog # SA1021) with DAB as the chromogen.

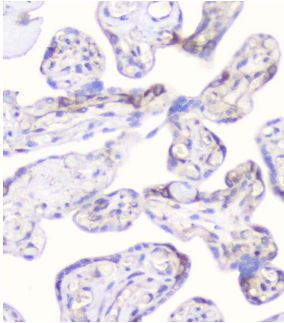


Figure 4. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).

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 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

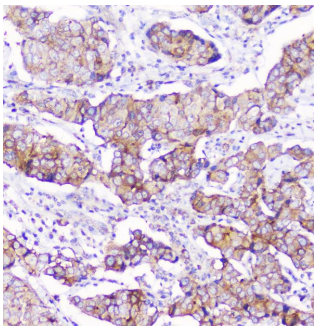


Figure 5. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).

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 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

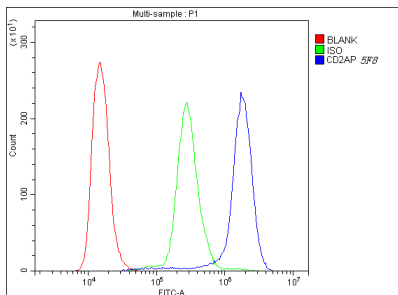


Figure 6. Flow Cytometry analysis of K562 cells using anti-CD2AP antibody (M01756).

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-CD2AP Antibody (M01756, 1ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

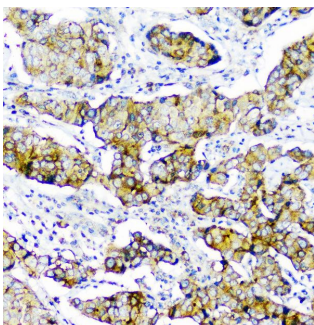


Figure 7. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).

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 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

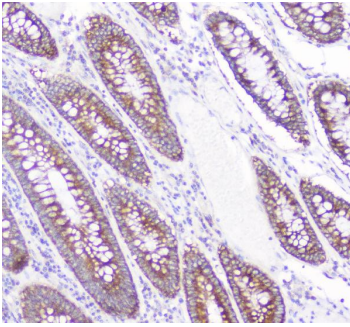


Figure 8. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).

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 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

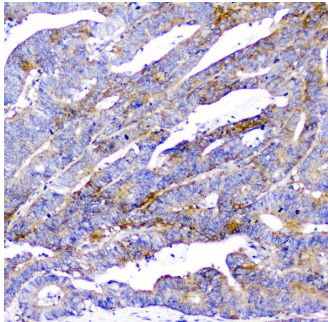


Figure 9. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).

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 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

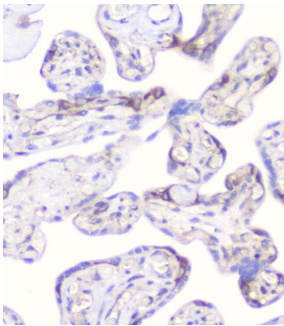


Figure 10. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).

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 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

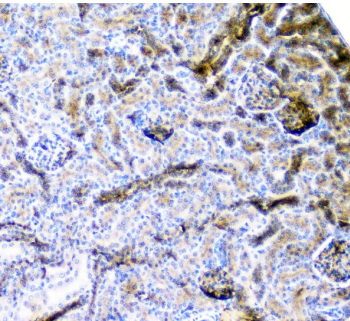
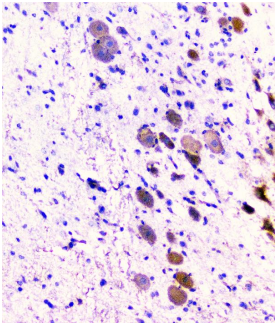


Figure 11. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).

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 1ug/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 Streptavidin-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 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

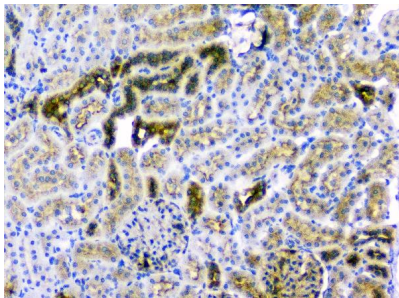


Figure 13. IHC analysis of CD2AP using anti-CD2AP antibody (M01756).

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 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

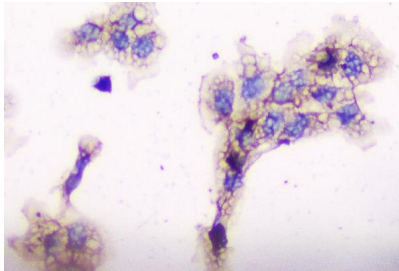


Figure 14. ICC analysis of CD2AP using anti-CD2AP antibody (M01756).

CD2AP was detected in immunocytochemical section of A431 cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 1ug/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 section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

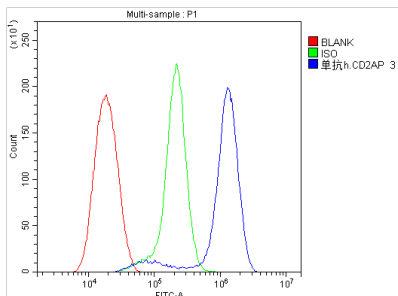
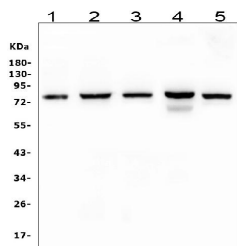


Figure 15. Flow Cytometry analysis of U2OS cells using anti-CD2AP antibody (M01756).

Overlay histogram showing U2OS cells stained with M01756 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-CD2AP Antibody (M01756, 1ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Figure 16. 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: rat liver tissue lysate,
Lane 2: rat testicular tissue lysate,
Lane 3: mouse pancreas tissue lysate,
Lane 4: mouse testicular tissue lysate,
Lane 5: mouse RAW246.7 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 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-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.

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