

## Product Datasheet

### CD2AP Mouse Monoclonal Antibody (orb507557)

<b>Catalog Number</b>	orb507557
<b>Category</b>	Antibodies
<b>Description</b>	Anti-CD2AP Antibody (monoclonal, 5F8). Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
<b>Target</b>	CD2-associated protein
<b>Clonality</b>	Monoclonal
<b>Species/Host</b>	Mouse
<b>Isotype</b>	Mouse IgG1
<b>Conjugation</b>	Unconjugated
<b>Reactivity</b>	Human, Mouse, Rat
<b>Form/Appearance</b>	Lyophilized
<b>Concentration</b>	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
<b>Buffer/Preservatives</b>	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na <sub>2</sub> HPO <sub>4</sub> .
<b>Reconstitution</b>	Add 0.2ml of distilled water will yield a concentration of 500µg/ml.
<b>Purification</b>	Immunogen affinity purified.
<b>Immunogen</b>	E. coli-derived human CD2AP recombinant protein (Position: K253-K337).
<b>UniProt ID</b>	<b>Q9Y5K6</b>
<b>MW</b>	80 kDa

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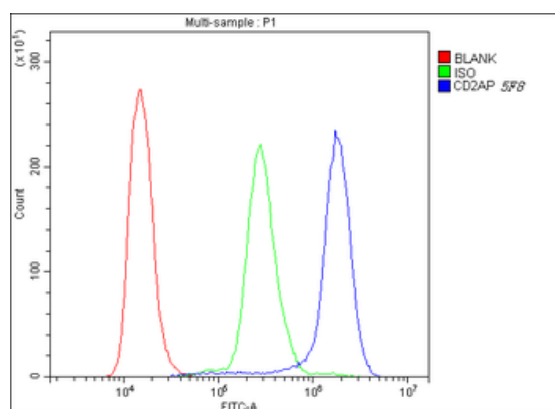
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<b>Tested applications</b>	FC, ICC, IF, IHC, WB
<b>Dilution range</b>	Western blot, 0.1-0.5µg/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1µg/ml Immunocytochemistry/Immunofluorescence, 5 µg/ml Flow Cytometry (Fixed), 1-3µg/1x10 <sup>6</sup> cells
<b>Specificity</b>	No cross reactivity with other proteins.
<b>Cross Reactivity</b>	No cross-reactivity with other proteins.
<b>Antibody Type</b>	Primary Antibody
<b>Clone Number</b>	5F8
<b>Storage</b>	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
<b>Note</b>	For research use only
<b>Expiration Date</b>	12 months from date of receipt.



Flow Cytometry analysis of K562 cells using anti-D2AP antibody. Overlay histogram showing K562 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-D2AP Antibody (1 µg/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (5-10 µg/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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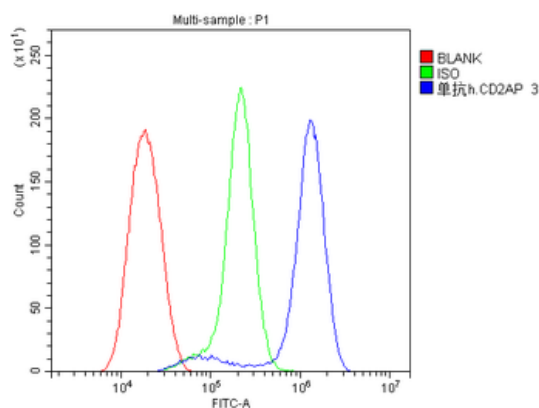
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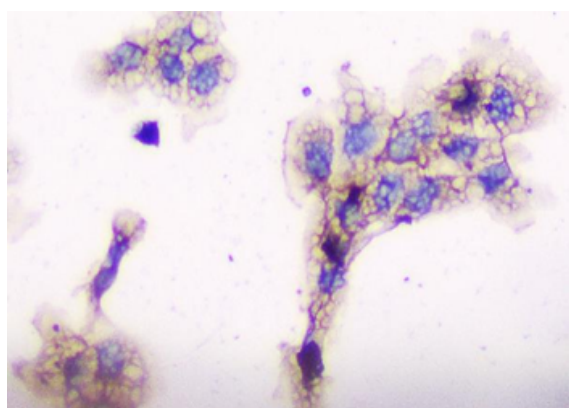
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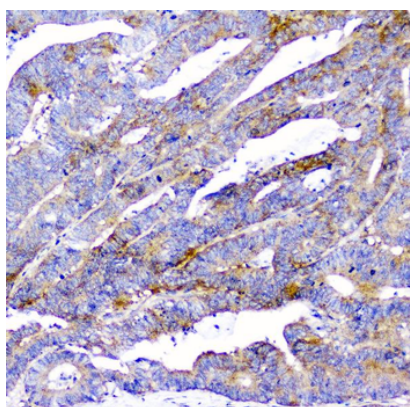
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Flow Cytometry analysis of U2OS cells using anti-CD2AP antibody. Overlay histogram showing U2OS cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-CD2AP Antibody (1  $\mu\text{g}/1 \times 10^6$  cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (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 rabbit IgG (1  $\mu\text{g}/1 \times 10^6$ ) used under the same conditions. Unlabelled sample (Red line) was also used as a control.



ICC analysis of CD2AP using anti-CD2AP antibody. CD2AP was detected in immunocytochemical section of A431 cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 1  $\mu\text{g}/\text{ml}$  mouse anti-CD2AP Antibody 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) with DAB as the chromogen.



IHC analysis of CD2AP using anti-CD2AP antibody. 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  $\mu\text{g}/\text{ml}$  mouse anti-CD2AP Antibody 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) with DAB as the chromogen.

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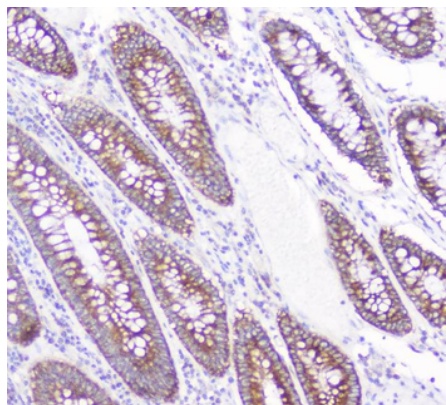
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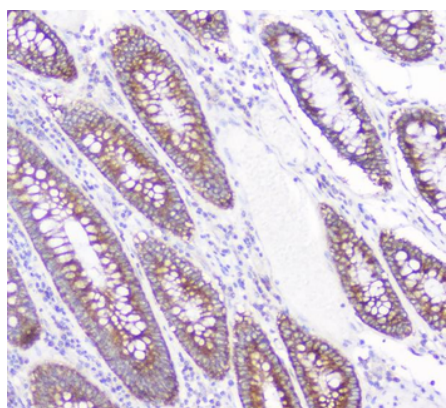
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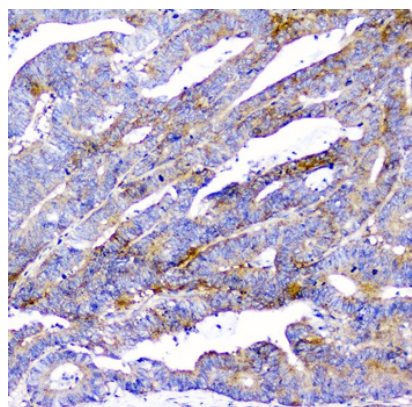
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IHC analysis of CD2AP using anti-CD2AP antibody. CD2AP was detected in paraffin-embedded section of human colon cancer. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section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



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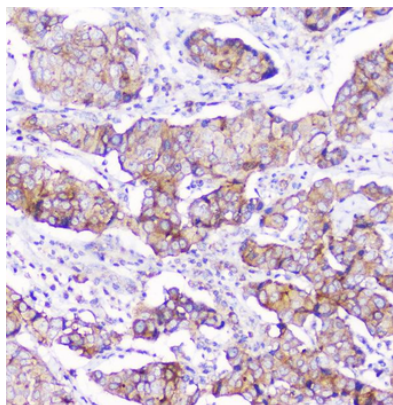
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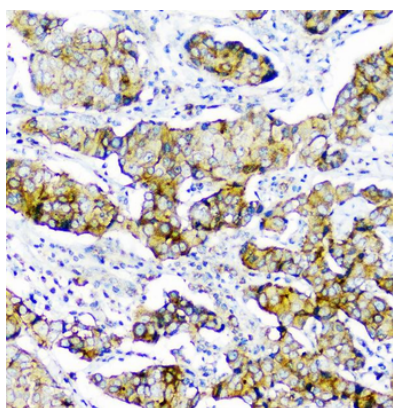
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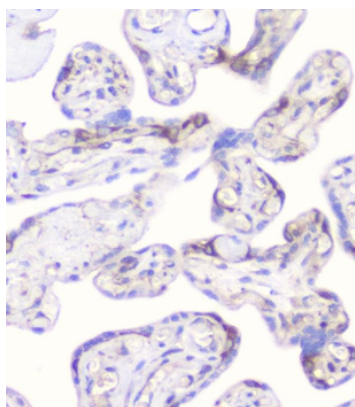
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IHC analysis of CD2AP using anti-CD2AP antibody. CD2AP was detected in paraffin-embedded section of human mammary cancer. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1  $\mu$ g/ml mouse anti-CD2AP Antibody 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) with DAB as the chromogen.



IHC analysis of CD2AP using anti-CD2AP antibody. 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  $\mu$ g/ml mouse anti-CD2AP Antibody 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) with DAB as the chromogen.



IHC analysis of CD2AP using anti-CD2AP antibody. 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  $\mu$ g/ml mouse anti-CD2AP Antibody 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) with DAB as the chromogen.

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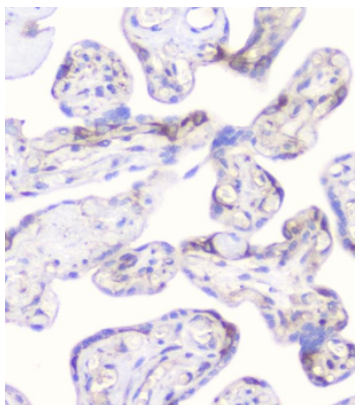
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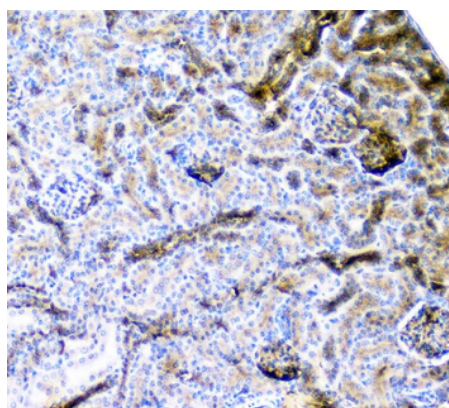
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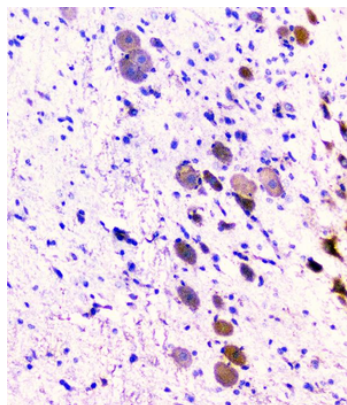
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IHC analysis of CD2AP using anti-CD2AP antibody. CD2AP was detected in paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1  $\mu$ g/ml mouse anti-CD2AP Antibody 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) with DAB as the chromogen.



IHC analysis of CD2AP using anti-CD2AP antibody. 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  $\mu$ g/ml mouse anti-CD2AP Antibody 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) with DAB as the chromogen.



IHC analysis of CD2AP using anti-CD2AP antibody. 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  $\mu$ g/ml mouse anti-CD2AP Antibody 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) with DAB as the chromogen.

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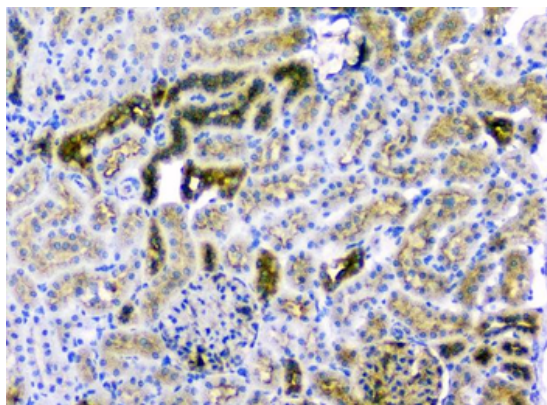
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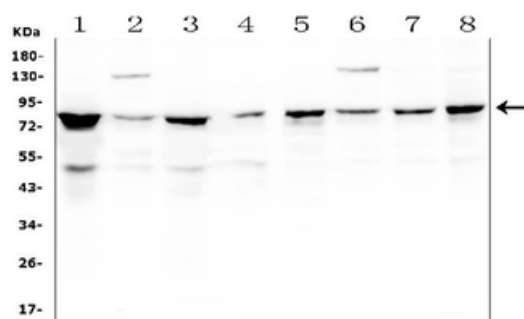
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Western blot analysis of CD2AP using anti-CD2AP antibody. Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50 µg of sample under reducing conditions. 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 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 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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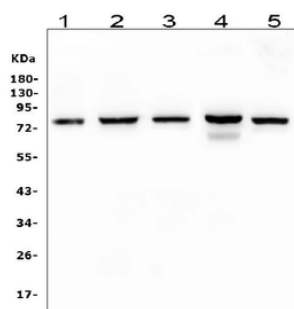
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