

## Product Datasheet

### Anti-CD44 Antibody (orb1972567)

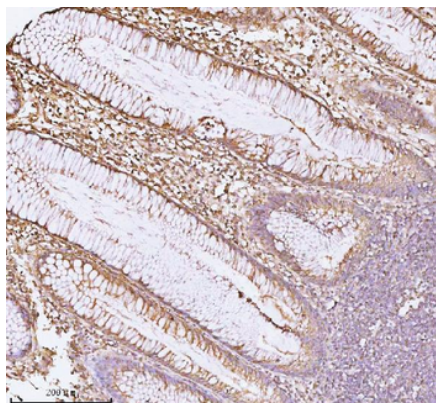
<b>Description</b>	Anti-CD44 Antibody. Tested in WB, IHC, ICC/IF, Flow Cytometry, ELISA applications. This antibody reacts with Human, Mouse, Rat.
<b>Species/Host</b>	Rabbit
<b>Reactivity</b>	Human, Mouse, Rat
<b>Conjugation</b>	Unconjugated
<b>Tested Applications</b>	ELISA, FC, ICC, IF, IHC, WB
<b>Immunogen</b>	E.coli-derived human CD44 recombinant protein (Position: Q21-E518). Human CD44 shares 66.5% amino acid (aa) sequence identity with mouse CD44.
<b>Form/Appearance</b>	Lyophilized
<b>Concentration</b>	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
<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>Application notes</b>	Western blot, 0.25-0.5 µg/ml, Human Immunohistochemistry, 2-5 µg/ml, Human, Mouse, Rat Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human Flow Cytometry (Fixed), 1-3 µg/1x10 <sup>6</sup> cells, Human ELISA, 0.1-0.5 µg/ml, -. Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml
<b>Clonality</b>	Polyclonal
<b>MW</b>	85 kDa
<b>Uniprot ID</b>	<b>P16070</b>
<b>Expiration Date</b>	12 months from date of receipt.

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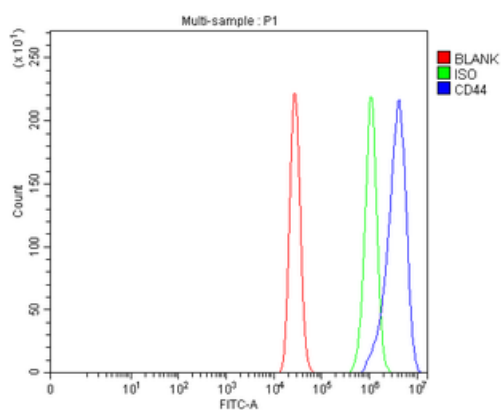
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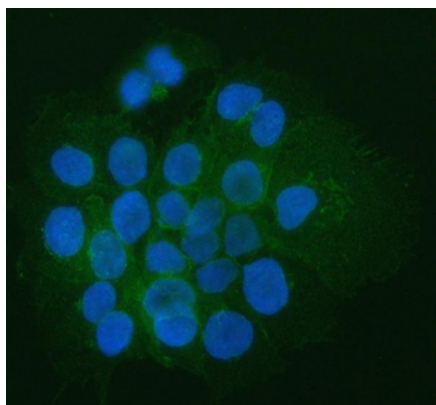
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IHC analysis of CD44 using anti-CD44 antibody. CD44 was detected in a paraffin-embedded section of human colon tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 μg/ml rabbit anti-CD44 Antibody 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 with DAB as the chromogen.



Flow Cytometry analysis of MCF-7 cells using anti-CD44 antibody. Overlay histogram showing MCF-7 cells (Blue line). The cells were fixed with 4% paraformaldehyde and blocked with 10% normal goat serum. And then incubated with rabbit anti-CD44 Antibody (1 μg/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit 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 rabbit IgG (1 μg/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.



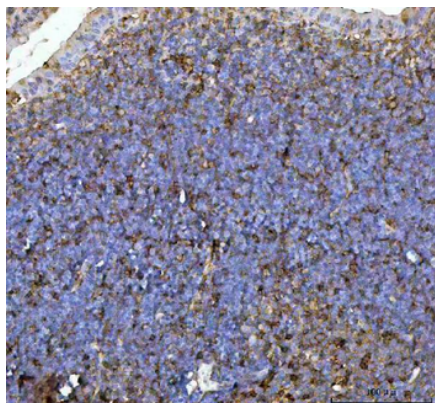
IF analysis of CD44 using anti-CD44 antibody. CD44 was detected in an immunocytochemical section of A431 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 μg/mL rabbit anti-CD44 Antibody overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

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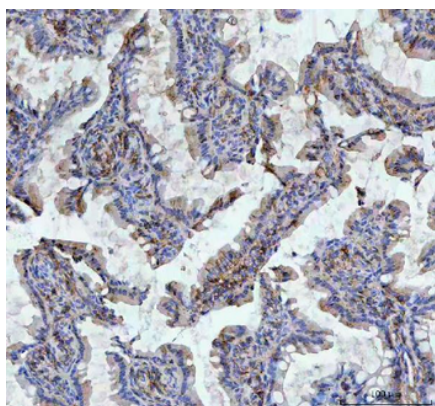
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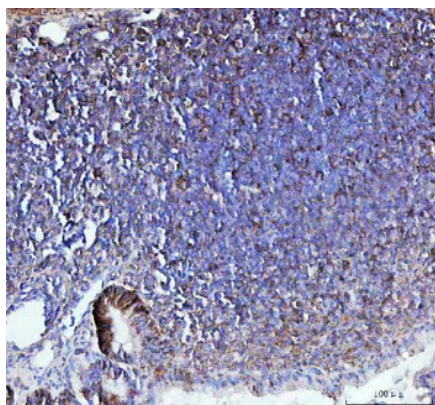
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IHC analysis of CD44 using anti-CD44 antibody. CD44 was detected in a paraffin-embedded section of mouse lymphaden tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-CD44 Antibody 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 with DAB as the chromogen.



IHC analysis of CD44 using anti-CD44 antibody. CD44 was detected in a paraffin-embedded section of rat colon tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-CD44 Antibody 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 with DAB as the chromogen.



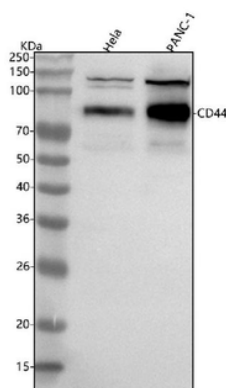
IHC analysis of CD44 using anti-CD44 antibody. CD44 was detected in a paraffin-embedded section of rat colon tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-CD44 Antibody 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 with DAB as the chromogen.

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Western blot analysis of CD44 using anti-CD44 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 30 ug of sample under reducing conditions. Lane 1: human HeLa whole cell lysates, Lane 2: human PANC-1 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-CD44 antigen affinity purified polyclonal antibody at 0.5  $\mu$ g/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 with Tanon 5200 system. A specific band was detected for CD44 at approximately 85 kDa. The expected band size for CD44 is at 82 kDa.

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