

## Product Datasheet

### EHD3 Rabbit Polyclonal Antibody (orb745908)

<b>Catalog Number</b>	orb745908
<b>Category</b>	Antibodies
<b>Description</b>	Anti-EHD3 Antibody. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Monkey, Mouse, Rat.
<b>Target</b>	EH domain-containing protein 3
<b>Clonality</b>	Polyclonal
<b>Species/Host</b>	Rabbit
<b>Isotype</b>	Rabbit IgG
<b>Conjugation</b>	Unconjugated
<b>Reactivity</b>	Human, Monkey, Mouse, Rat
<b>Form/Appearance</b>	Lyophilized
<b>Concentration</b>	500 µg/ml
<b>Buffer/Preservatives</b>	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na <sub>2</sub> HPO <sub>4</sub> .
<b>Reconstitution</b>	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.
<b>Purification</b>	Immunogen affinity purified.
<b>Immunogen</b>	A synthetic peptide corresponding to a sequence at the C-terminus of human EHD3, which shares 93.8% and 100% amino acid (aa) sequence identity with mouse and rat EHD3, respectively.
<b>UniProt ID</b>	<b>Q9NZN3</b>

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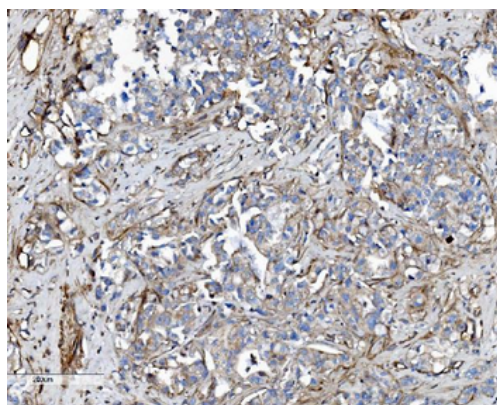
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<b>MW</b>	70 kDa
<b>Tested applications</b>	FC, ICC, IF, IHC, WB
<b>Dilution range</b>	Western blot, 0.1-0.25µg/ml, Human, Monkey, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5µg/ml, Human, Mouse, Rat Immunocytochemistry/Immunofluorescence, 5µg/ml, Human Flow Cytometry (Fixed), 1-3µg/1x10 <sup>6</sup> cells, Human
<b>Cross Reactivity</b>	No cross-reactivity with other proteins.
<b>Antibody Type</b>	Primary Antibody
<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.



IHC analysis of EHD3 using anti-EHD3 antibody. EHD3 was detected in paraffin-embedded section of human gastric adenocarcinoma 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-EHD3 Antibody overnight at 4°C. Biotinylated goat anti-rabbit 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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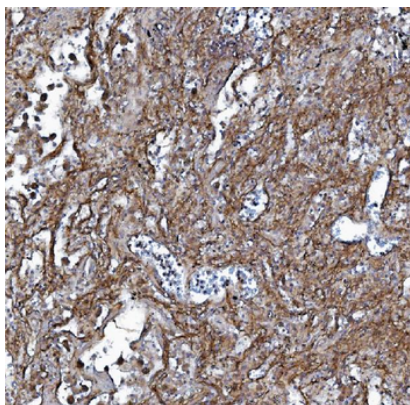
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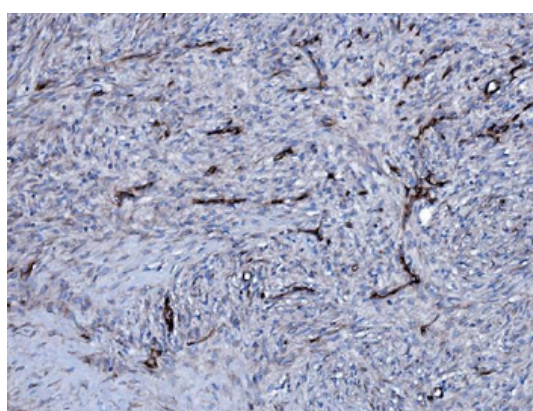
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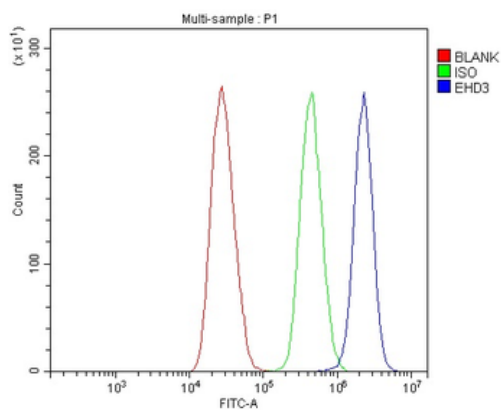
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IHC analysis of EHD3 using anti-EHD3 antibody. EHD3 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-EHD3 Antibody overnight at 4°C. Biotinylated goat anti-rabbit 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 EHD3 using anti-EHD3 antibody. EHD3 was detected in paraffin-embedded section of human ovarian serous adenocarcinoma 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-EHD3 Antibody overnight at 4°C. Biotinylated goat anti-rabbit 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.



Flow Cytometry analysis of MCF-7 cells using anti-EHD3 antibody. Overlay histogram showing MCF-7 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 rabbit anti-EHD3 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 without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

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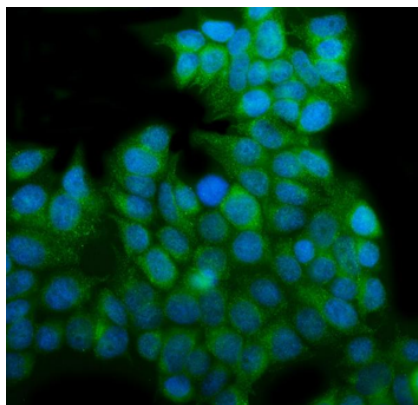
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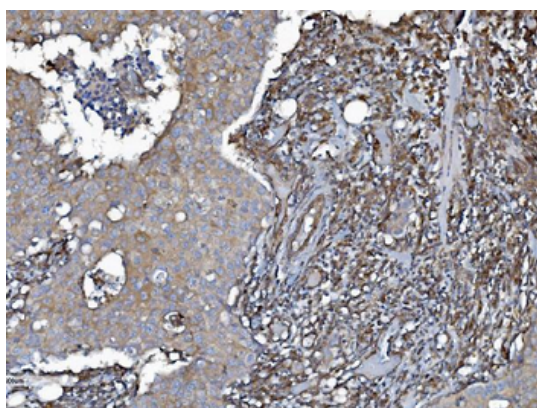
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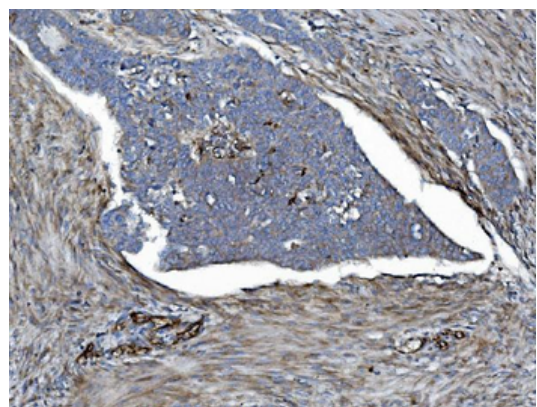
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IF analysis of EHD3 using anti-EHD3 antibody. EHD3 was detected in immunocytochemical section of MCF-7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5  $\mu\text{g}/\text{mL}$  rabbit anti-EHD3 Antibody overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:100 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.



IHC analysis of EHD3 using anti-EHD3 antibody. EHD3 was detected in paraffin-embedded section of human breast cancer 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  $\mu\text{g}/\text{ml}$  rabbit anti-EHD3 Antibody overnight at 4°C. Biotinylated goat anti-rabbit 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 EHD3 using anti-EHD3 antibody. EHD3 was detected in paraffin-embedded section of human ovarian serous adenocarcinoma 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  $\mu\text{g}/\text{ml}$  rabbit anti-EHD3 Antibody overnight at 4°C. Biotinylated goat anti-rabbit 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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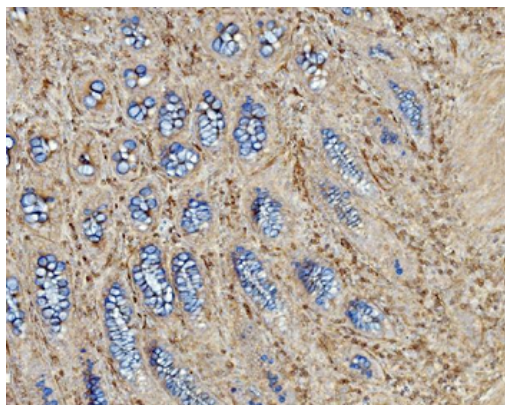
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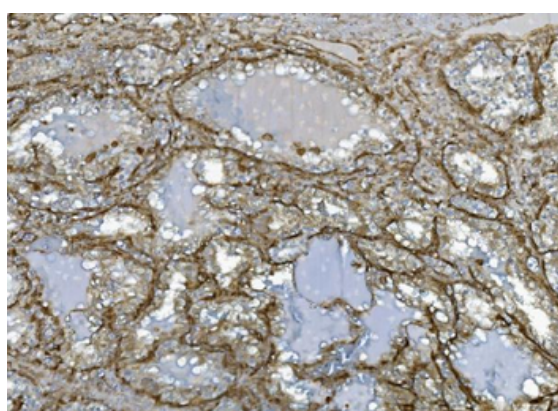
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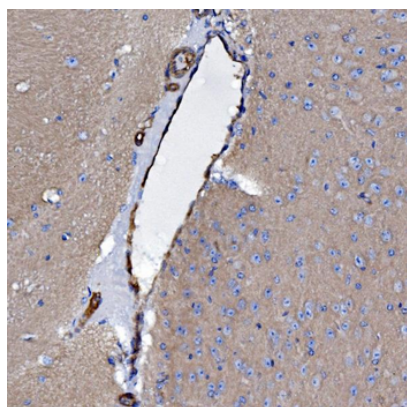
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IHC analysis of EHD3 using anti-EHD3 antibody. EHD3 was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-EHD3 Antibody overnight at 4°C. Biotinylated goat anti-rabbit 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 EHD3 using anti-EHD3 antibody. EHD3 was detected in paraffin-embedded section of human renal clear cell carcinoma 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-EHD3 Antibody overnight at 4°C. Biotinylated goat anti-rabbit 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 EHD3 using anti-EHD3 antibody. EHD3 was detected in paraffin-embedded section of mouse brain 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-EHD3 Antibody overnight at 4°C. Biotinylated goat anti-rabbit 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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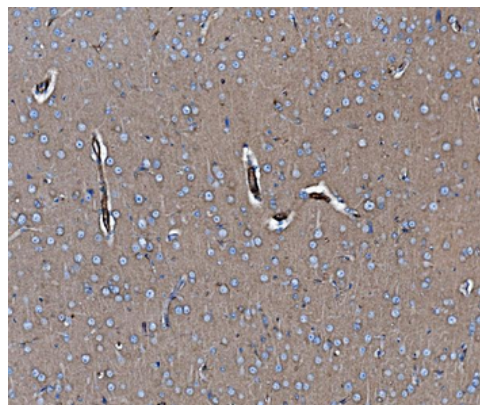
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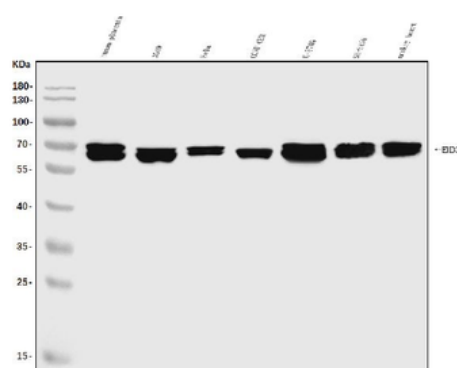
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IHC analysis of EHD3 using anti-EHD3 antibody. EHD3 was detected in paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-EHD3 Antibody overnight at 4°C. Biotinylated goat anti-rabbit 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.



Western blot analysis of EHD3 using anti-EHD3 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 µg of sample under reducing conditions. Lane 1: human placenta tissue lysates, Lane 2: human A549 whole cell lysates, Lane 3: human Hela whole cell lysates, Lane 4: human CCRF-CEM whole cell lysates, Lane 5: human U-87MG whole cell lysates, Lane 6: human SY-SY5Y whole cell lysates, Lane 7: monkey heart tissue 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-EHD3 antigen affinity purified polyclonal antibody at 0.25 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-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 EHD3 at approximately 70 KD. The expected band size for EHD3 is at 70 KD.

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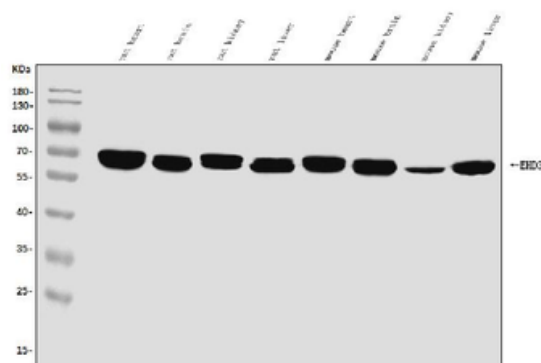
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