

## Anti-EHD3 Antibody Picoband®

Catalog Number: A04576-1

### About EHD3

Eps15 homology domain-containing protein 3, abbreviated as EDH3 and also known as PAST3, is a protein encoded by the EHD3 gene. The Eps15 homology (EH) domain-containing protein family consists of four members, EHD1, EHD2, EHD3, and EHD4. The chromosomal locations of the human EHD genes are as follows: EHD1 maps to 11q13, EHD2 maps to 19q13.3, EHD3 maps to 2p21, and EHD4 maps to 15q11.1 (1-3). The encoded proteins of all EHD family members contain multiple conserved regions, which include an amino-terminal nucleotide-binding consensus site, a bipartite nuclear localization signal, and a carboxy-terminal EH protein-binding domain with an EF-hand motif (3,4). EHD1 is ubiquitously expressed with increased expression in testis (2,3). EHD2, EHD3, and EHD4 have more specific expression with EHD2 highly expressed in heart, EHD3 expressed in brain, kidney, liver, placenta, ovary, and heart, and EHD4 expressed in heart, placenta, and pancreas (1,5,6). The EHD proteins may participate in ligand-induced endocytosis (3-5).

### Overview

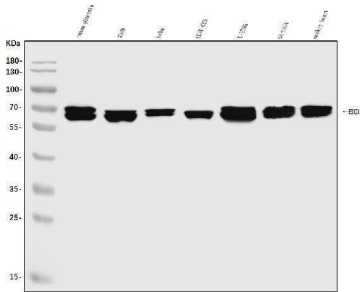
Product Name	Anti-EHD3 Antibody Picoband®
Reactive Species	Human, Monkey, Mouse, Rat
Description	Boster Bio Anti-EHD3 Antibody Picoband® catalog # A04576-1. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Monkey, Mouse, Rat. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na <sub>2</sub> HPO <sub>4</sub> .
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	Rabbit
Uniprot ID	Q9NZN3

### Technical Details

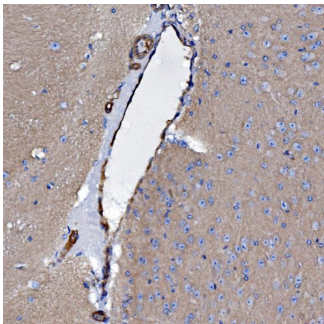
Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human EHD3, which shares 93.8% and 100% amino acid (aa) sequence identity with mouse and rat EHD3, respectively.
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.

Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.1-0.25ug/ml, Human, Monkey, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human, Mouse, Rat Immunocytochemistry/Immunofluorescence, 5ug/ml, Human Flow Cytometry (Fixed), 1-3ug/1x10 <sup>6</sup> cells, Human

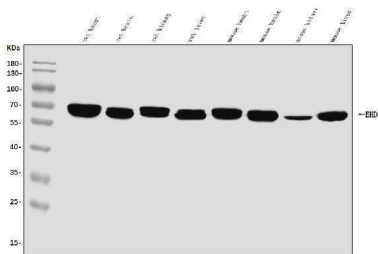
## Anti-EHD3 Antibody Picoband® (A04576-1) Images



Western blot analysis of EHD3 using anti-EHD3 antibody (A04576-1). 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 30ug 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 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 rabbit anti-EHD3 antigen affinity purified polyclonal antibody (Catalog # A04576-1) at 0.25 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-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 (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for EHD3 at approximately 70KD. The expected band size for EHD3 is at 70KD.

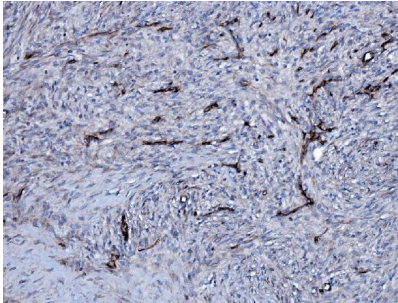


IHC analysis of EHD3 using anti-EHD3 antibody (A04576-1). EHD3 was detected in paraffin-embedded section of mouse brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-EHD3 Antibody (A04576-1) 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) (Catalog # SA1022) with DAB as the chromogen.

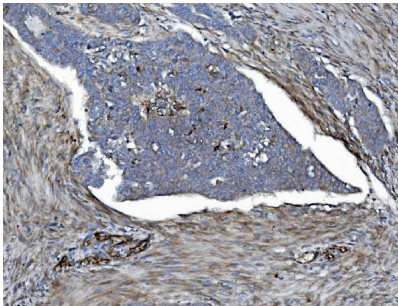


Western blot analysis of EHD3 using anti-EHD3 antibody (A04576-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30ug of sample under reducing conditions. Lane 1: rat heart tissue lysates, Lane 2: rat brain tissue lysates, Lane 3: rat kidney tissue lysates, Lane 4: rat liver tissue lysates, Lane 5: mouse heart tissue lysates, Lane 6: mouse brain tissue lysates, Lane 7: mouse kidney tissue lysates, Lane 8: mouse liver tissue lysates. 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 rabbit anti-EHD3 antigen affinity purified polyclonal antibody (Catalog # A04576-1) at 0.25 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-rabbit IgG-HRP secondary

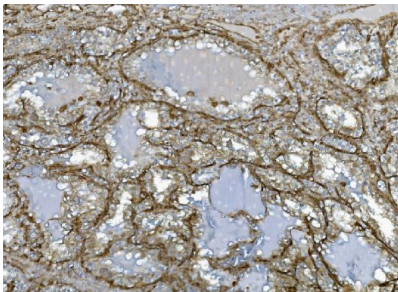
antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for EHD3 at approximately 70KD. The expected band size for EHD3 is at 70KD.



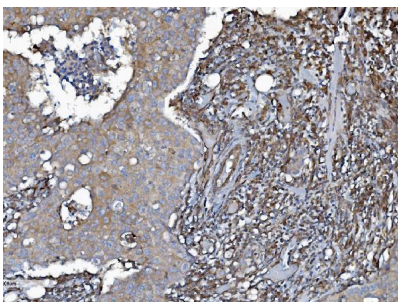
IHC analysis of EHD3 using anti-EHD3 antibody (A04576-1). EHD3 was detected in paraffin-embedded section of human ovarian serous adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-EHD3 Antibody (A04576-1) 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) (Catalog # SA1022) with DAB as the chromogen.



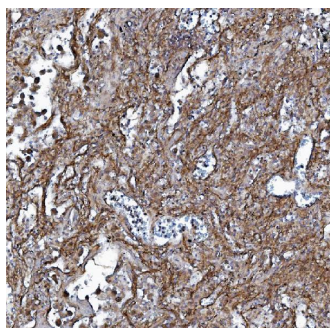
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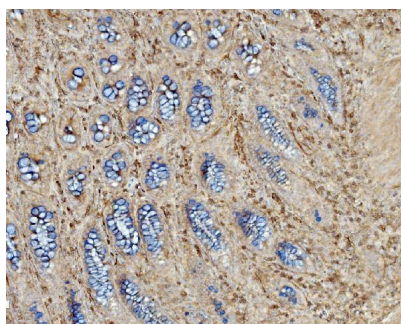
IHC analysis of EHD3 using anti-EHD3 antibody (A04576-1). EHD3 was detected in paraffin-embedded section of human renal clear cell carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-EHD3 Antibody (A04576-1) 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) (Catalog # SA1022) with DAB as the chromogen.



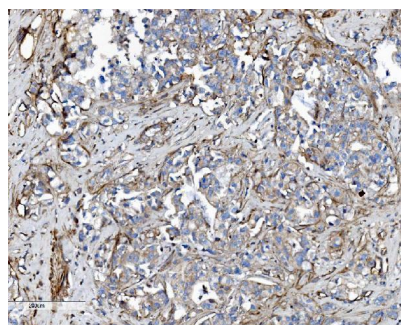
IHC analysis of EHD3 using anti-EHD3 antibody (A04576-1). EHD3 was detected in paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-EHD3 Antibody (A04576-1) 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) (Catalog # SA1022) with DAB as the chromogen.



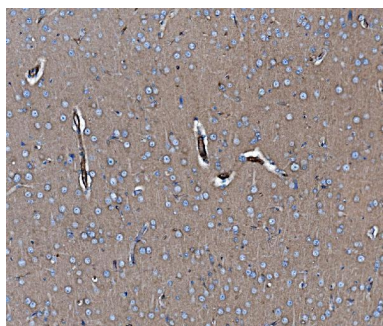
IHC analysis of EHD3 using anti-EHD3 antibody (A04576-1). EHD3 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-EHD3 Antibody (A04576-1) 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) (Catalog # SA1022) with DAB as the chromogen.



IHC analysis of EHD3 using anti-EHD3 antibody (A04576-1). 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 2ug/ml rabbit anti-EHD3 Antibody (A04576-1) 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) (Catalog # SA1022) with DAB as the chromogen.

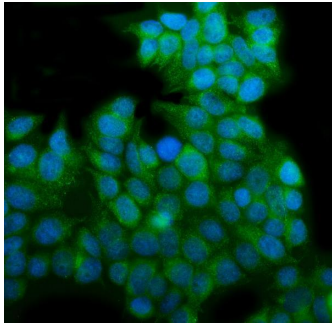


IHC analysis of EHD3 using anti-EHD3 antibody (A04576-1). EHD3 was detected in paraffin-embedded section of human gastric adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-EHD3 Antibody (A04576-1) 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) (Catalog # SA1022) with DAB as the chromogen.

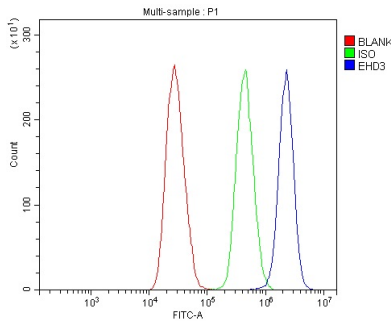


IHC analysis of EHD3 using anti-EHD3 antibody (A04576-1). EHD3 was detected in paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-EHD3 Antibody (A04576-1) 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) (Catalog # SA1022) with DAB as the chromogen.

IF analysis of EHD3 using anti-EHD3 antibody (A04576-1). EHD3 was detected in immunocytochemical section of MCF-7 cells. 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 5ug/mL rabbit anti-EHD3 Antibody



(A04576-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) 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.



Flow Cytometry analysis of MCF-7 cells using anti-EHD3 antibody (A04576-1). Overlay histogram showing MCF-7 cells stained with A04576-1 (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 (A04576-1, 1ug/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/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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### Anti-EHD3 Antibody

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