BOSTER
antibody and ELISA experts

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Anti-Aspartate beta hydroxylase/ASPH Antibody Picoband®

Catalog Number: PB9478

About ASPH

ASPH is also known as Aspartyl/asparaginyl beta-hydroxylase. This gene is thought to play an important role in calcium homeostasis. And the gene is expressed from two promoters and undergoes extensive alternative splicing. The encoded set of proteins share varying amounts of overlap near their N-termini but have substantial variations in their C-terminal domains resulting in distinct functional properties. The longest isoforms (a and f) include a C-terminal Aspartyl/Asparaginyl beta-hydroxylase domain that hydroxylates aspartic acid or asparagine residues in the epidermal growth factor (EGF)-like domains of some proteins, including protein C, coagulation factors VII, IX, and X, and the complement factors C1R and C1S. Other isoforms differ primarily in the C-terminal sequence and lack the hydroxylase domain, and some have been localized to the endoplasmic and sarcoplasmic reticulum. Some of these isoforms are found in complexes with calsequestrin, triadin, and the ryanodine receptor, and have been shown to regulate calcium release from the sarcoplasmic reticulum. Some isoforms have been implicated in metastasis.

Overview

Product Name	Anti-Aspartate beta hydroxylase/ASPH Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-Aspartate beta hydroxylase/ASPH Antibody Picoband® catalog # PB9478. Tested in Flow Cytometry, IHC, ICC, WB applications. This antibody reacts with Human, 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, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains antibody formulated with stabilizing components, 0.9 mg NaCl, 0.2 mg Na2HPO4, and 0.05 mg NaN3. *This antibody is supplied in a stabilized formulation. Compatibility with conjugation reactions depends on the chemistry of the conjugation method used. For conjugation methods that are not compatible with the stabilizing components present in this formulation, a carrier-free antibody format is required.
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	Q12797

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human ASPH, identical to the related mouse sequence.

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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, 0.1-0.5ug/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human Immunocytochemistry, 0.5-1ug/ml, Human Flow Cytometry (Fixed), 1-3ug/1x10^6 cells, Human

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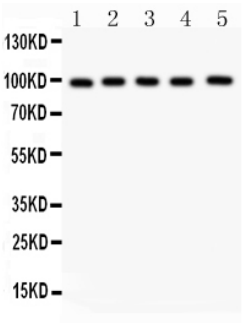
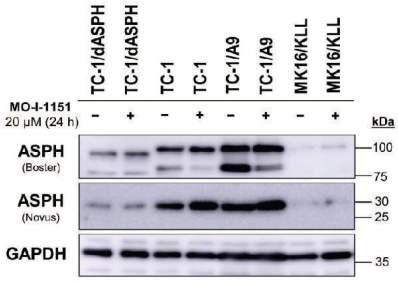
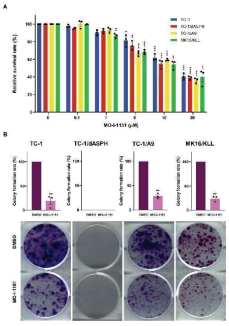
Anti-Aspartate beta hydroxylase/ASPH Antibody Picoband® (PB9478) Images



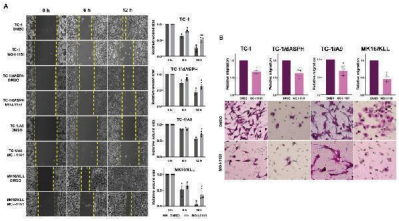
Western blot analysis of ASPH using anti-ASPH antibody (PB9478). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions. Lane 1: Rat Brain Tissue Lysate, Lane 2: Rat Liver Tissue Lysate, Lane 3: HELA Whole Cell Lysate, Lane 4: HEPG2 Whole Cell Lysate, Lane 5: HEPA 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 rabbit anti-ASPH antigen affinity purified polyclonal antibody (Catalog # PB9478) 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-rabbit 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 # EK1002) with Tanon 5200 system. A specific band was detected for ASPH at approximately 100KD. The expected band size for ASPH is at 86KD.



Immunoblotting detection of the ASPH protein. TC-1, TC-1/dASPH, TC-1/A9 and MK16/KLL cells were treated with 20 uM MO-I-1151 inhibitor for 24 h and DMSO was used as a control. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. Antibodies applied for ASPH staining are indicated.Index in PubMed under a CC BY license. PMID: 38356711



The effect of ASPH inhibition on cell proliferation. (A) TC-1, TC-1/dASPH, TC-1/A9 and MK16/KLL cells were treated with the MO-I-1151 inhibitor at concentration 0.1, 1, 5, 10 and 20 uM for 48 h and then subjected to an MTT assay or (B) incubated with MO-I-1151 at 20 uM for 7 days, stained with crystal violet and photographed. Images were quantified by ImageJ software. DMSO was used as a control. Data represents the mean ± SEM of three independent experiments. Statistical significance refers to the comparison with the non-treated (DMSO) samples. * p



The effect of ASPH inhibition on cell migration. (A) Confluent TC-1, TC-1/dASPH, TC-1/A9 and MK16/KLL cells were incubated with 20 uM MO-I-1151 for the indicated times. The migration was measured by the area of the wound made in cells and images were taken at 0, 6 and 12 h and images were quantified using ImageJ software. (B) Cell lines were treated with 20 uM MO-I-1151 in transwell chambers. After 24 h, the cells were fixed and stained. Microscopic images were quantified by ImageJ software. DMSO was used as a

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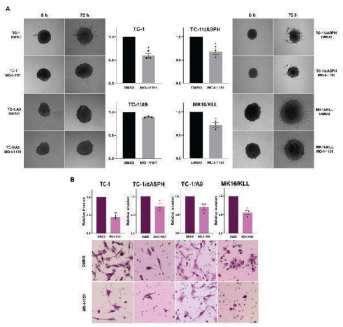
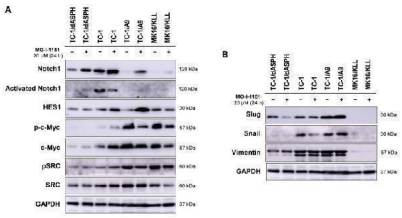
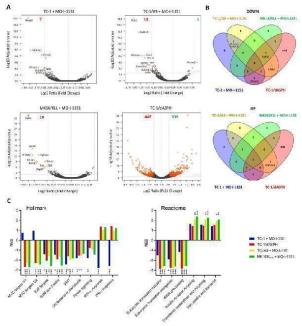
control. Data represents the mean ± SEM of three independent experiments. Statistical significance refers to the comparison with the non-treated (DMSO) samples. * p



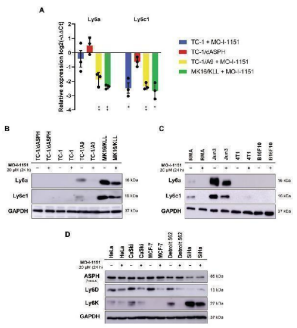
The effect of ASPH inhibition on cell invasions. (A) Spheroids were embedded into a 3D collagen matrix and treated with 20 uM MO-I-1151. Images were taken at 0 and 72 h and quantified by ImageJ software. (B) Cell lines were treated with 20 uM MO-I-1151 in pre-coated Matrigel transwell chambers. After 24 h, the cells were fixed and stained. Microscopic images were quantified by ImageJ software. DMSO was used as a control. Data represents the mean ± SEM of three independent experiments. Statistical significance refers to the comparison with the non-treated (DMSO) samples. * p



The effect of ASPH inhibition on cellular signaling. Cells were treated with 20 uM MO-I-1151 for 24 h (DMSO was used as a control), and protein samples were collected and subjected to SDS-PAGE electrophoresis and immunoblotting. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. (A) Notch and SRC pathways, (B) epithelial-mesenchymal pathway.Index in PubMed under a CC BY license. PMID: 38356711



The effect of ASPH downregulation on gene expression. Bulk RNA-seq was performed after ASPH inhibition with 20 uM MO-I-1151 inhibitor for 24 h in TC-1, TC-1/A9, and MK16/KLL cells or ASPH deactivation with CRISPR/Cas9 in TC-1/dASPH cells (n=3). DMSO was used as a control . (A) Differential gene expression. Orange dots indicate genes with at least 2-fold decreased or increased expression and p adj ≤0.01. Numbers of these genes are indicated in green and red colors, respectively. (B) Overlap of down- and upregulated genes. (C) Enrichment analysis. Some of the most significant differences found with Hallmark and Reactome gene sets are shown.Index in PubMed under a CC BY license. PMID: 38356711



The effect of ASPH inhibition on regulation of Ly6 family members. Cells were treated with 20 uM MO-I-1151 inhibitor for 24 h and DMSO was used as a control. (A) Ly6a and Ly6c1 expression was detected by RT-qPCR and relative quantification was calculated. Data represents the mean ± SEM of three independent experiments. TC-1, TC-1/A9, and MK16/KLL cell lines incubated with MO-I-1151 were compared with DMSO-treated controls and TC-1/dASPH cells with TC-1 cell line. * p

IHC analysis of ASPH using anti-ASPH antibody (PB9478). ASPH was detected in paraffin-embedded section of Human Mammary Cancer Tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with

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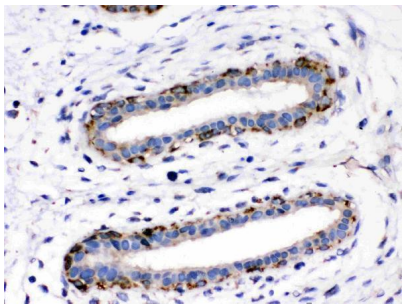
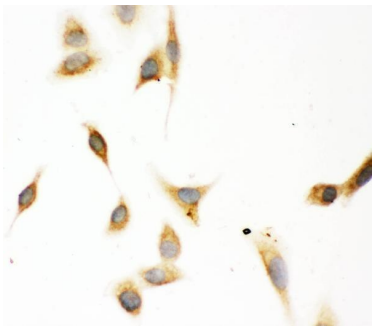
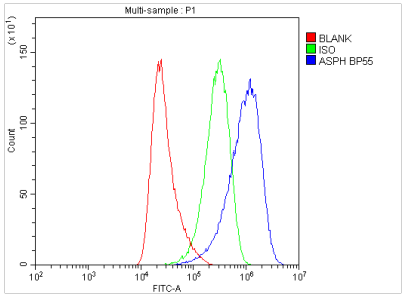


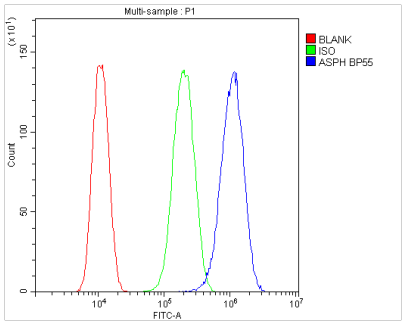
10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-ASPH Antibody (PB9478) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.



IHC analysis of ASPH using anti-ASPH antibody (PB9478). ASPH was detected in immunocytochemical section of A549 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 rabbit anti-ASPH Antibody (PB9478) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.



Flow Cytometry analysis of HeLa cells using anti-ASPH antibody (PB9478). Overlay histogram showing HeLa cells stained with PB9478 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-ASPH Antibody (PB9478,1ug/1x106 cells) for 30 min at 20°C. DyLight488 conjugated goat anti-rabbit IgG (BA1127, 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.



Flow Cytometry analysis of U87 cells using anti-ASPH antibody (PB9478). Overlay histogram showing U87 cells stained with PB9478 (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-ASPH Antibody (PB9478,1ug/1x106 cells) for 30 min at 20°C. DyLight488 conjugated goat anti-rabbit IgG (BA1127, 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 without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

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