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Anti-BCAT1 Antibody Picoband®

Catalog Number: PA1538

About BCAT1

BCAT1, Branched-chain Aminotransferase1, is also know as BCT1. The BCAT1 gene is highly expressed early in embryogenesis, and during organogenesis its expression is localized to the neural tube, the somites, and the mesonephric tubules. The gene is also expressed in several MYC-based tumors. The BCAT1 gene is mapped to chromosome 12. Lack of the enzyme BCT can cause auxotroph, a kind of auxotrophic mutant in Chinese-hamster ovary cells that lacks the ability to grow if alpha-ketoisovaleric acid, alpha-ketoisocaproic acid, and alpha-keto-beta-methylvaleric acid are substituted for valine, leucine, and isoleucine in the culture medium.

Overview

Product Name	Anti-BCAT1 Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-BCAT1 Antibody catalog # PA1538. Tested in Flow Cytometry, IHC, 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, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.
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	P54687

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human BCAT1, different from the related mouse sequence by one amino acid, rat sequence by three amino acids.
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).
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG
Form	Lyophilized

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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), 2-5ug/ml, Mouse, Rat Flow Cytometry(Fixed), 1-3 ug/1x10^6 cells, Human

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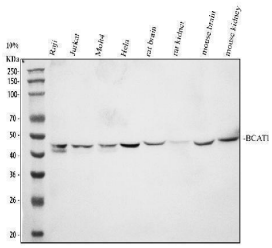
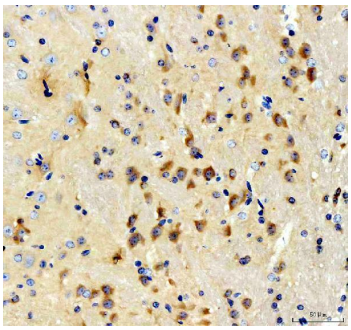
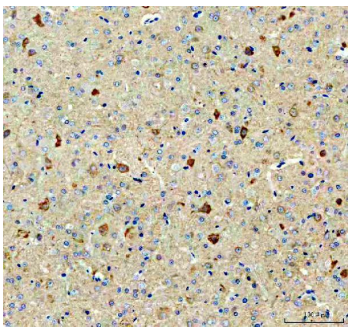
Anti-BCAT1 Antibody Picoband® (PA1538) Images



Western blot analysis of BCAT1 using anti-BCAT1 antibody (PA1538). Electrophoresis was performed on a 10% SDS-PAGE gel at 80V (Stacking gel) / 120V (Resolving gel) for 2 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human Raji whole cell lysates, Lane 2: human Jurkat whole cell lysates, Lane 3: human MOLT-4 whole cell lysates, Lane 4: human Hela whole cell lysates, Lane 5: rat brain tissue lysates, Lane 6: rat kidney tissue lysates, Lane 7: mouse brain tissue lysates, Lane 8: mouse kidney 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-BCAT1 antigen affinity purified polyclonal antibody (Catalog # PA1538) 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 1:5000 for 1.5 hour at RT. The signal is developed using an ECL Plus Western Blotting Substrate (Catalog # AR1196-200) with Tanon 5200 system. A specific band was detected for BCAT1 at approximately 43 kDa. The expected band size for BCAT1 is at 43 kDa.



IHC analysis of BCAT1 using anti-BCAT1 antibody (PA1538). BCAT1 was detected in a paraffin-embedded section of mouse brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-BCAT1 Antibody (PA1538) 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 (Catalog # SV0002) with DAB as the chromogen.



IHC analysis of BCAT1 using anti-BCAT1 antibody (PA1538). BCAT1 was detected in a paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-BCAT1 Antibody (PA1538) 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 (Catalog # SV0002) with DAB as the chromogen.

Flow Cytometry analysis of Jurkat cells using anti-BCAT1 antibody (PA1538). Overlay histogram showing Jurkat cells stained with PA1538 (Blue line). To facilitate intracellular

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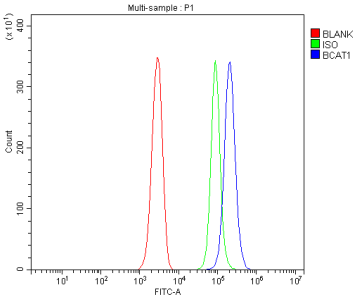
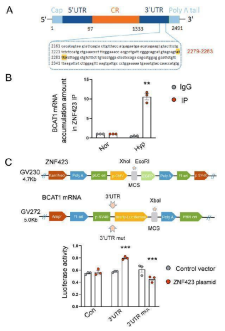
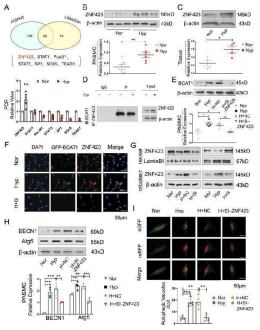


staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-BCAT1 Antibody (PA1538, 1 ug/1x106 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x106 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x106) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



ZNF423 maintaines the stable expression of BCAT1 by binding the AU-rich elements (AREs) of the 3′-UTR of BCAT1 mRNA in hypoxic PASMCs. a The binding sites for ZNF423 in the 3′-UTR of BCAT1 mRNA. b The correlation between ZNF423 and BCAT1 mRNA was detected by real-time PCR after RNA immunoprecipitation (RIP) (n = 3). c Reporter constructs containing luciferase, and the 3′-UTR of BCAT1 mRNA and mutated 3′-UTR of BCAT1 mRNA were used to estimate the activity of various luciferase reporter genes (n = 3). Nor normoxia, Hyp hypoxia, Con con083 control vector, 3′-UTR 3′-UTR luciferase reporter plasmid, 3′-UTR mut 3′-UTR ARE mutant luciferase reporter plasmid. Statistical analysis was performed with two-way ANOVA. All values are presented as the mean ± SEM. ** p



Hypoxia leads to the transfer of ZNF423 from the nucleus to the cytoplasm, where it bound BCAT1 to promote autophagy activity. a Bioinformatics analysis of proteins associated with BCAT1. Upside: According to the JASPAR database and LASAGNA-Search 2.0 database, there was 28 genes that may bind to bcat1, and the binding ability of ZNF423, STAT1, Pou5f1, STAT3, SP1, SOX9, and TEAD1 was strong. Underside: RT-PCR analysis of the mRNA levels of ZNF423, STAT1, Pou5f1, STAT3, SP1, SOX9, and TEAD1 with rat beta-actin serving as the standard in PASMCs under NOR or HYP for 24 h (n = 5). b Western blot analysis of the expression of ZNF423 in PASMCs under NOR or HYP for 24 h (n = 6). c ZNF423 protein levels were assayed in pulmonary arterial tissues of hypoxic model rats (n = 4). d Coimmunoprecipitation of whole-cell lysates of PASMCs exposed to normoxia or hypoxia for 24 h with anti-ZNF423, followed by probing with anti-BCAT1 (n = 3). e Western blot analysis of BCAT1 expression in PASMCs transfected with ZNF423 siRNA under NOR or HYP for 24 h (n = 4). f PASMCs were exposed to HYP for 24 h, and the colocalization between BCAT1 and ZNF423 was determined by immunofluorescence. GFP-BCAT1 (green), ZNF423 (red), and DAPI (blue). Scale bar = 50 um (n = 3). g The translocation of ZNF423 between the nucleus and cytoplasm in PASMCs transfected with BCAT1 siRNA or gabapentin (n = 3). h Western blot analysis of the expression of BECN1 and Atg5 in PASMCs transfected with ZNF423 siRNA under HYP for 24 h (n = 4). i Autophagic flux of PASMCs cotransfected with eGFP-mRFP-LC3 plasmid and control siRNA or ZNF423 siRNA under HYP for 24 h. Scale bar = 50 um (n = 5). Nor normoxia, Hyp hypoxia, H + G hypoxia plus gabapentin, H +

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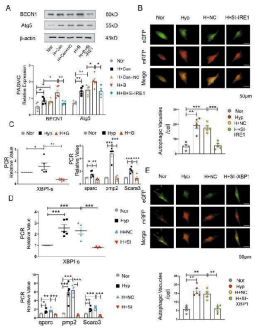
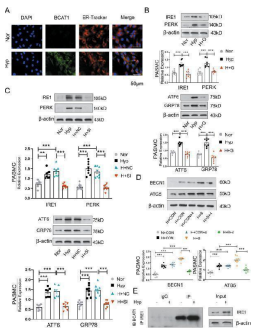
NC hypoxia plus control siRNA, H + SI hypoxia plus BCAT1 siRNA, H + si-ZNF423 hypoxia plus ZNF423 siRNA, IP immunoprecipitation, IB immunoblotting. Statistical analysis was performed with one-way ANOVA or the Student’s t test. All values are presented as the mean ± SEM. * p



BCAT1 regulates autophagy during hypoxia by activating ERs via the IRE1-XBP1-RIDD axis. a Western blot analysis of BECN1 and Atg5 in PASMCs cotransfected with BCAT1 and IRE1 siRNA (n = 5). b Autophagic flux was monitored in PASMCs cotransfected with eGFP-mRFP-LC3 plasmid and control siRNA or IRE1 siRNA that were then exposed to HYP for 24 h. Scale bar = 50 um (n = 3). c , d RT-PCR analysis of the mRNA levels of XBP1-s, sparc, pmp2, and Scara3 with rat beta-actin serving as the standard (n = 5). e The formation of autophagosomes was detected, and autophagic activity was estimated in cells in which the expression of XBP1 was knocked down with XBP1 siRNA under HYP for 24 h. Scale bar = 50 μm (n = 5). Nor normoxia, Hyp hypoxia, H + G hypoxia plus gabapentin, H + NC hypoxia plus control siRNA, H + SI hypoxia plus BCAT1 siRNA, H + SI-IRE1 hypoxia plus IRE1 siRNA, H + SI-XBP1 hypoxia plus XBP1 siRNA, H + Con hypoxia plus control vector, H + B hypoxia plus BCAT1 plasmid, H + Con+NC hypoxia plus control vector plus control siRNA, H + B + Si-IRE hypoxia plus BCAT1 plasmid plus IRE1 siRNA. Statistical analysis was performed with one-way ANOVA. All values are presented as the mean ± SEM. * p



BCAT1 regulates autophagy through the endoplasmic reticulum stress pathway. a Expression of BCAT1 and ER-Tracker Red staining in PASMCs exposed to NOR or HYP for 24 h. Scale bar = 50 um (n = 3). b Western blot analysis of PERK, IRE1, ATF6, and GRP78 protein expression in the ERs pathway in PASMCs treated with gabapentin (n = 8). c Western blot analysis of IRE1, PERK, ATF6, and GRP78 expression in PASMCs transfected with BCAT1 siRNA (n = 8). d Western blot analysis of BECN1 and Atg5 in PASMCs treated with the ERs pathway inhibitor 4-PBA and BCAT1 plasmid (n = 8). e Coimmunoprecipitation of the whole-cell lysates of PASMCs exposed to normoxia or hypoxia for 24 h with anti-IRE1, followed by probing with anti-BCAT1 (n = 3). Nor normoxia, Hyp hypoxia, H + G hypoxia plus gabapentin, H + NC hypoxia plus control siRNA, H + SI hypoxia plus BCAT1 siRNA, N + Con normoxia plus control vector, H + Con hypoxia plus control vector, H + B hypoxia plus BCAT1 plasmid, H + Con+4 hypoxia plus control vector plus 4-phenylbutyric acid, H + B + 4 hypoxia plus BCAT1 plasmid plus 4-phenylbutyric acid, IP immunoprecipitation, IB immunoblotting. Statistical analysis was performed with one-way ANOVA. All values are presented as the mean ± SEM. ** p

Upregulation of BCAT1 expression induced by hypoxia leads to PASMC autophagy. a Western blot analysis of BECN1 and Atg5 protein expression in PASMCs treated with the inhibitor gabapentin (20 μM) (n = 8). b Western blot analysis of

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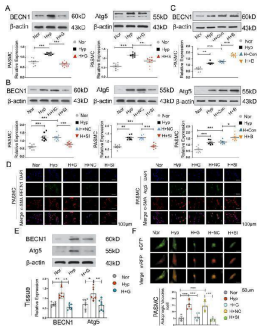
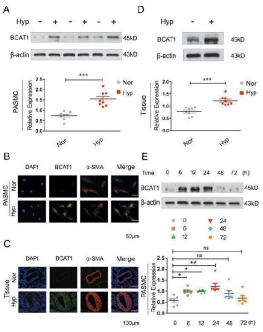


BECN1 and Atg5 protein expression in PASMCs transfected with BCAT1 siRNA or BCAT1 plasmid (n = 8). c , d Immunofluorescence staining for BECN1 and Atg5 in PASMCs. BECN1 and Atg5 (green), alpha-SMA (red), and DAPI (blue). Scale bar = 50 um (n = 3). e Western blot analysis of BECN1 and Atg5 expression in the pulmonary arterial tissues of hypoxia model rats treated with gabapentin (n = 7). f Measurement of autophagic flux in PASMCs transfected with eGFP-mRFP-LC3 plasmid and exposed under NOR or HYP for 24 h treated with BCAT1 siRNA or the BCAT1 inhibitor gabapentin. Yellow and red dots indicate autolysosomes and autophagosomes, respectively. Scale bar = 50 um (n = 6). Nor normoxia, Hyp hypoxia, Mct monocrotaline, H + G hypoxia plus gabapentin, M + G monocrotaline plus gabapentin, H + NC hypoxia plus control siRNA, H + SI hypoxia plus BCAT1 siRNA, H + Con hypoxia plus control vector, H + B hypoxia plus BCAT1 plasmid. Statistical analysis was performed with one-way ANOVA. All values are presented as the mean ± SEM. * p



Hypoxia resultes in the increased expression of BCAT1. a Western blot analysis of BCAT1 expression in hypoxic PASMCs (n = 8). b Subcellular distribution of BCAT1 in PASMCs determined by immunofluorescence analysis. Scale bars: 50 um (n = 3). c The cellular expression of BCAT1 in the smooth muscle layer of lung tissues from hypoxic model rats determined by immunofluorescence staining analysis. Scale bar = 100 um (n = 3). d BCAT1 protein levels in pulmonary arterial tissues of hypoxia model rats (n = 8). e Time course of BCAT1 expression of PASMCs at 0, 6, 12, 24, 48, and 72 h after hypoxia treatment (n = 6). Nor normoxia, Hyp hypoxia, Mct monocrotaline. Statistical analysis was performed with one-way ANOVA or the Student’s t test. All values are presented as the mean ± SEM. * p

Anti-BCAT1 Antibody

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