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antibody and ELISA experts

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Anti-PAH Antibody Picoband®

Catalog Number: A00761-1-carrier-free

About PAH

Phenylalanine hydroxylase (PAH) is an enzyme that catalyzes the hydroxylation of the aromatic side-chain of phenylalanine to generate tyrosine. It is one of three members of the biopterin-dependent aromatic amino acid hydroxylases, a class of monooxygenase that uses tetrahydrobiopterin (BH4, a pteridine cofactor) and a non-heme iron for catalysis. Deficiency of this enzyme activity results in the autosomal recessive disorder phenylketonuria.

Overview

Product Name	Anti-PAH Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-PAH Antibody Picoband® catalog # A00761-1. Tested in Flow Cytometry(Intracellular), 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	P00439

Technical Details

Immunogen	E. coli-derived human PAH recombinant protein (Position: R71-H208). Human PAH shares 89.1% and 88.4% amino acid (aa) sequence identity with mouse and rat PAH, 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).
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.

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Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.1-0.5ug/ml, Human, Mouse, Rat, Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human, Mouse, Rat Flow Cytometry (Fixed), 1-3 ug/1x10^6 cells, Human

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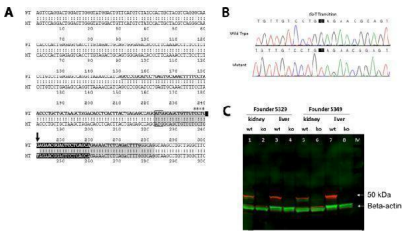
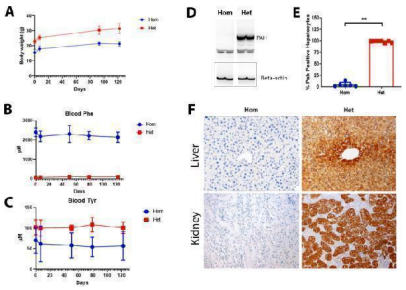
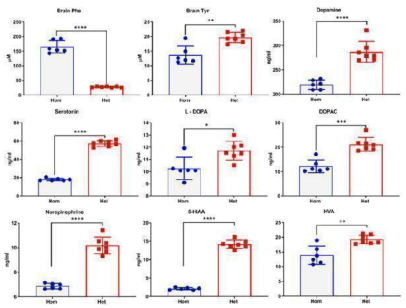
Anti-PAH Antibody Picoband® (A00761-1-carrier-free) Images



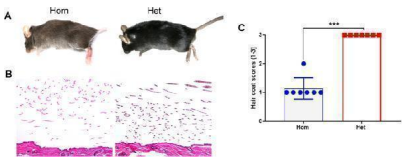
Creation of a Pah -KO mouse model. (A) The mouse genomic locus on chromosome 10 and the murine targeted allele after CRISPR gene editing are shown. Exon 1 contains the translation initiation codon. NCBI GeneID: 18478; RefSeq transcript: NM_008777. Exon 1 sequence is shadowed gray, the start codon (ATG) is framed, gRNA is shown as white letters on a black background, the asterisks and arrow indicate the position of PAM and G>T transition. The donor oligo for CRISPR targeting is in bold and underlined. (B) Sequencing genomic DNA samples of the founder 5349. The point mutation of G to T is indicated in the black boxes. (C) Western blot analyses to detect PAH protein in liver or kidney of wildtype (wt) or Hom (ko) mice. Solubilized proteins (60 µg) from mouse liver or kidney were fractionated and immune-blotted with an anti-PAH antibody. The PAH protein (red; expected size 50 kDa) is evident in wildtype mice (lanes 1, 3, 5 and 7) but is absent in Hom mice (2, 4, 6 and 8); lanes 1, 2, 5, 6) are from kidney; lanes 3, 4, 7, 8 are from liver. Equal loading is indicated by probing with antibody to beta-actin (green). M, molecular weight marker lane (bands not visible in this image). Index in PubMed under a CC BY license. PMID: 33790381



Characterization of Hom and Het Pah -KO mice for 6 months. (A) The Hom mice showed lower bodyweights than the Het mice throughout the study (B) Summary of blood Phe at various timepoints is shown (n = 7/group) (C) Summary of blood Tyr at various timepoints is shown (n = 7/group). The graphical data are represented as mean ± SD (Phe and Tyr levels in Hom vs Het mice p



Analysis of Phe, Tyr and neurotransmitters in brains of Hom and Het Pah -KO mice at 6 months. Scatter plot charts show brain Phe, Tyr, serotonin, dopamine, L-DOPA, DOPAC, NE, 5-HIAA, and HVA. n = 6 Hom, 7 Het. Bars indicate group mean and the error bars indicate standard deviation (* p = 0.01, ** p = 0.003—0.007, *** p = 0.0001, **** p



Hair coat color in Hom- Pah KO mice. The difference in hair coat color is grossly visible (A). Hair coat of the Hom mouse is light brown whereas it is dark brown for the Het mouse. (B) Hair shafts from the Hom mouse contain less melanin pigment when compared to the Het mouse. H&E stain 20× (C) Summary of hair melanin scores is shown in the scatter plot graph. n = 7 Hom, 7 Het. Bars indicate group median score and the error bars indicate range (*** p = 0.0006). Index in PubMed under a CC BY license. PMID: 33790381

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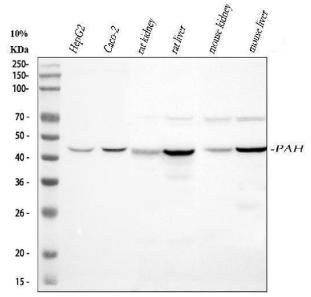
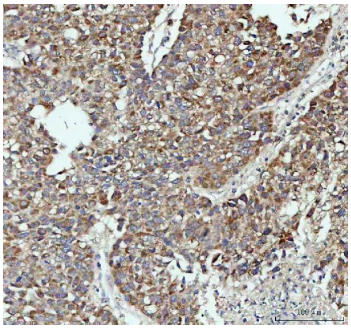
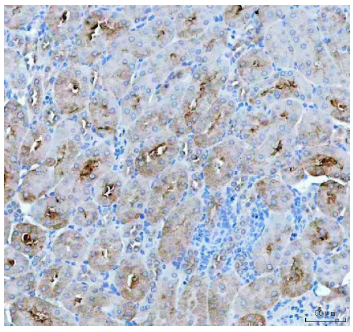


Western blot analysis of PAH using anti-PAH antibody (A00761-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human HepG2 whole cell lysates, Lane 2: human CACO-2 whole cell lysates, Lane 3: rat kidney tissue lysates, Lane 4: rat liver tissue lysates, Lane 5: mouse kidney tissue lysates, Lane 6: mouse liver 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-PAH antigen affinity purified polyclonal antibody (Catalog # A00761-1) 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 Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for PAH at approximately 45 kDa. The expected band size for PAH is at 52 kDa.



IHC analysis of PAH using anti-PAH antibody (A00761-1). PAH was detected in a paraffin-embedded section of human liver cancer 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-PAH Antibody (A00761-1) 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 PAH using anti-PAH antibody (A00761-1). PAH was detected in a paraffin-embedded section of mouse kidney 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-PAH Antibody (A00761-1) 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 PAH using anti-PAH antibody (A00761-1). PAH was detected in a paraffin-embedded section of rat kidney 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

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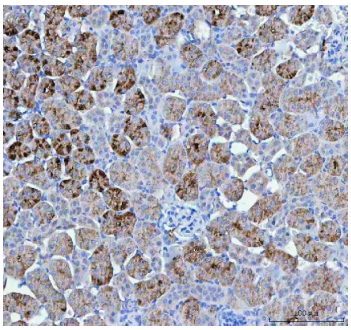
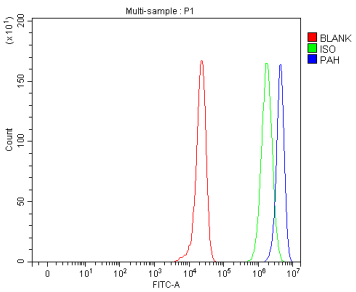


rabbit anti-PAH Antibody (A00761-1) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.



Flow Cytometry analysis of HepG2 cells using anti-PAH antibody (A00761-1). Overlay histogram showing HepG2 cells stained with A00761-1 (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-PAH Antibody (A00761-1, 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 (Red line) was also used as a control.

2 Publications Citing This Product

1. PubMed ID: 10.1038/s41598-021-86663-8, CRISPR/Cas9 generated knockout mice lacking phenylalanine hydroxylase protein as a novel preclinical model for human phenylketonuria

2. PubMed ID: 33790381, Singh K,Cornell CS,Jackson R,Kabiri M,Phipps M,Desai M,Fogle R,Ying X,Anarat-Cappillino G,Geller S,Johnson J,Roberts E,Malley K,Devlin T,DeRiso M,Berthelette P,Zhang YV,Ryan S,Rao S,Thurberg BL,Bangari DS,Kyostio-Moore S.CRISPR/Cas9 generated knockout mice lacking phenylalanine hydroxylase protein as a novel preclinical model for human phenylketonuria.Sci Rep.2021 Mar 31;11(1):7254.doi:10.1038/s41598-021-86663-8.PMID:33790381.

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