BOSTER
antibody and ELISA experts

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Anti-SIAH Interacting Protein/CACYBP Antibody Picoband®

Catalog Number: RP1104

About CACYBP

Calcyclin-binding protein is a protein that in humans is encoded by the CACYBP gene. And this gene is mapped to 1q24-q25. The protein encoded by this gene is a calcyclin binding protein. It may be involved in calcium-dependent ubiquitination and subsequent proteosomal degradation of target proteins. In addition, it probably serves as a molecular bridge in ubiquitin E3 complexes and participates in the ubiquitin-mediated degradation of beta-catenin. Two alternatively spliced transcript variants encoding different isoforms have been found for this gene.

Overview

Product Name	Anti-SIAH Interacting Protein/CACYBP Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-SIAH Interacting Protein/CACYBP Antibody catalog # RP1104. Tested in Flow Cytometry, IP, IF, 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, IP, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains antibody formulated with stabilizing components, 0.9mg NaCl, 0.2mg Na2HPO4, 0.01mg 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	Q9HB71

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human CACYBP, different from the related mouse sequence by five amino acids, and from the related rat sequence by six 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) and ICC.

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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, Mouse, Rat Immunocytochemistry/Immunofluorescence,2ug/ml, Human Flow Cytometry (Fixed), 1-3ug/1x10^6 cells, Human Immunoprecipitation, 0.5-2 ug/ml, Human

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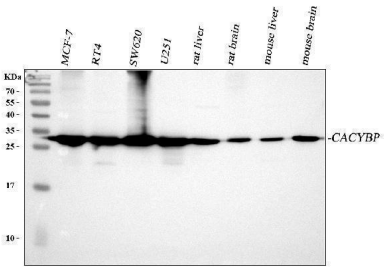
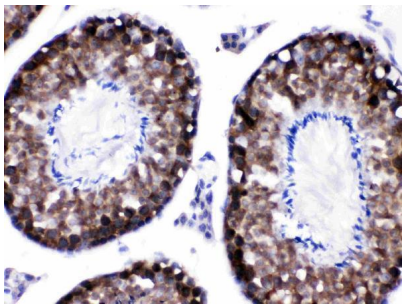
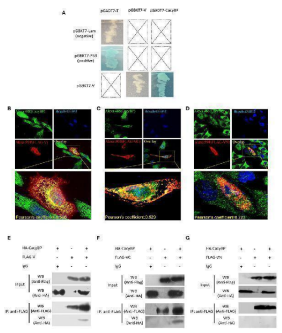
Anti-SIAH Interacting Protein/CACYBP Antibody Picoband® (RP1104) Images



Western blot analysis of CACYBP using anti-CACYBP antibody (RP1104). 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 MCF-7 whole cell lysates, Lane 2: human RT4 whole cell lysates, Lane 3: human SW620 whole cell lysates, Lane 4: human U251 whole cell lysates, Lane 5: rat liver tissue lysates, Lane 6: rat brain tissue lysates, Lane 7: mouse liver tissue lysates, Lane 8: mouse brain 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-CACYBP antigen affinity purified polyclonal antibody (Catalog # RP1104) 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 CACYBP at approximately 26 kDa. The expected band size for CACYBP is at 26 kDa.



IHC analysis of CACYBP using anti-CACYBP antibody (RP1104). CACYBP was detected in a paraffin-embedded section of mouse testis 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 1 ug/ml rabbit anti-CACYBP Antibody (RP1104) 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.



CacyBP bound to C-terminal domains of V protein. (A) Identification of protein interaction partners of V protein by yeast two-hybrid screening of a CEF cDNA library. (B–D) Co-localization of CacyBP with V, VC, and VN proteins in DF-1 cells. DF-1 cells were plated on coverslips and transfected with Flag-V, Flag-VN and Flag-VC. Forty-eight hours after transfection, the cells were stained with mouse anti-FLAG and rabbit anti-CacyBP antibodies, which was followed by staining with donkey anti-rabbit Alexa Fluor ® 488 (green) and goat anti-mouse Alexa Fluor ® ; 594 (Red) as secondary antibodies. The nucleus was subsequently stained with Hoechst 33342, and the images were captured using an ANDOR Revolution WD confocal microscope. Pearson's coefficient were analysis by Imaris (Microscopy Image Analysis Software, Bitplane, Switzerland), and Pearson's coefficient >0.5 were considered to be probable co-localization. (E–G) HEK-293T cells in 60 mm cell culture

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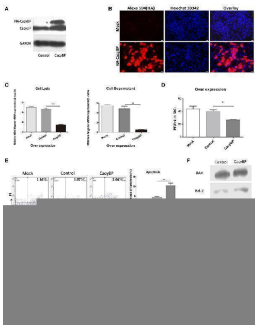
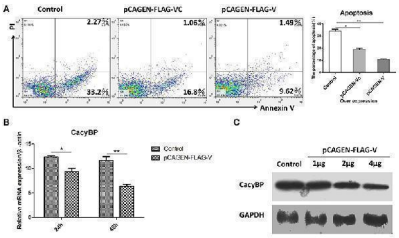
dishes were co-transfected with the pCAGEN-Flag-V (or Flag-VN or Flag-VC) and pCMV-HA-CacyBP expression plasmids. Transfected cells were harvested and lysed 48 h after transfection, and the experiment was conducted according to the manufacturer's instructions for the Co-Immunoprecipitation Kit. After washing, immunoprecipitated proteins were identified and analyzed by western blotting using anti-HA or anti-FLAG antibodies. PMID: 30234028



Overexpression of CacyBP in DF-1 cells arrested viral replication and enhanced apoptosis. DF-1 cells were transfected with mock and/or control and CacyBP/SIP and incubated for 48 h. Mock, treated with transfection reagent; control, transfected with pCMV-3HA; CacyBP, transfected with pCMV-3HA-CacyBP/SIP. (A) Endogenous and exogenous CacyBP/SIP was detected by western blotting. (B) Expression of the 3HA-CacyBP protein in DF-1 cells was detected using immunofluorescence. (C) Replication kinetics of NDV RNAs from DF-1 cells. Both cell lysates and supernatants were collected 48 h after transfection (at 24 h after transfection, 1 MOI of F48E9 NDV was inoculated in three groups). Q-PCR was used to measure viral RNA replication. (D) Viral plaque formation tests were further used to measure the number of virus particles in supernatants. (E) Flow cytometry was used to analyze cell apoptosis. DF-1 cells were transfected with mock and/or control and CacyBP/SIP and incubated for 48 h, an annexin V assay was followed by flow cytometry to monitor for percentage of cells undergoing early apoptosis (bottom right quadrant) and late apoptosis(upper right quadrant).Y-axis is PI signal;X-asis is annexin V-FITC signal, right graph data were the percentage of total apoptosis (bottom and upper right quadrant)and data from three independent experiments. (F) The transfected cell lysate was analyzed by western blotting with the indicated apoptosis-related antibody. (G) Q-PCR was used to analyze the expression of apoptosis-related markers and (H) immune-associated markers 24 h after transfection with pCMV-HA or pCMV-HA-CacyBP. Data shown in (C,D) are mean ± SD of four independent experiments in (E,G,H) are mean ± SD of three independent expriments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. PMID: 30234028



V protein inhibited apoptosis by downregulating CacyBP in DF-1 cells. DF-1 cells were transfected with the control (pCAGEN-Flag), pCAGEN-Flag-VC, and pCAGEN-Flag-V. (A) The transfected cells were washed and harvested 48 h after transfection, and flow cytometry was used to analyze cell apoptosis. (B) Twenty-four and forty-eight hours after transfection, the mRNA levels of CacyBP were measured by Q-PCR. (C) Protein expression of CacyBP was measured in DF-1 cells. The cells were transfected with pCAGEN-Flag (control) and pCAGEN-Flag-V (1, 2, and 4 ug), and after 48 h, the cells were harvested and analyzed by western blotting. Data shown in (A,B) are mean ± SD of three independent experiments. * $P < 0.05$; ** $P < 0.01$. PMID: 30234028

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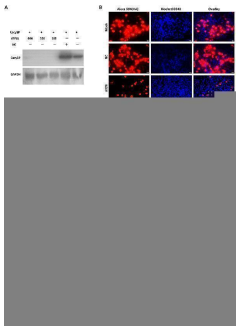
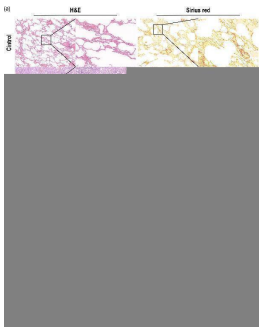
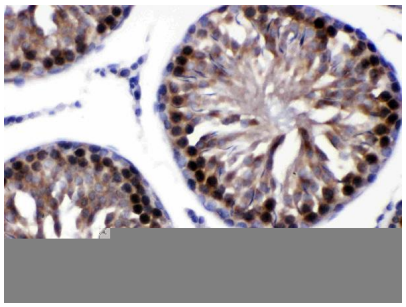


Knockdown of CacyBP facilitated viral replication and arrested apoptosis in DF-1 cells. DF-1 cells were transfected with mock and/or NC and si-CacyBP/SIP and incubated for 36 h. Mock, treated with transfection regent; NC, transfected with si-NC; si183/326/644, separately transfected with those siRNAs targets CacyBP/SIP. (A) Endogenous CacyBP/SIP was detected by western blotting. (B) DF-1 cells were co-transfected with si-CacyBP (326) and pCMV-HA-CacyBP. Thirty-six hours after transfection, protein expression of HA-CacyBP was detected by anti-HA antibody through immunofluorescence in DF-1 cells. (C) Replication kinetics of NDV RNAs from the mock, NC, and siRNA (326) groups of DF-1 cells; 1 MOI of F48E9 NDV were inoculated into the three groups of cells 24 h after transfection. Q-PCR was used to measure viral RNA replication 24 hpi. (D) Viral plaque formation tests were further used to measure the number of viruses in the supernatants. (E) Three cell cultures were prepared and transfected with mock, NC and siRNA (326), and then, the cells were washed and harvested, an annexin V assay was followed by flow cytometry to monitor for percentage of cells undergoing early apoptosis (bottom right quadrant) and late apoptosis(upper right quadrant). Y-axis is PI signal; X-asis is annexin V-FITC signal. Right graph data were the percentage of total apoptosis (bottom and upper right quadrant)and data from three independent experiments. (F–H) Q-PCR was used to analyze the expression of apoptosis-related markers and immune-associated markers 24 h after transfection with NC and si-CacyBP/SIP. (G) The transfected cell lysate was analyzed by western blotting with the indicated apoptosis-related antibody. Data shown in (C,D) are mean ± SD of four independent experiments, in (E,G,H) are mean ± SD of three independent expriments. * $P < 0.05$ and ** $P < 0.01$.Index in PubMed under a CC BY license. PMID: 30234028



(A) H&E and Sirius red staining revealed severe pulmonary fibrosis in the COVID-19 and PQ groups. (B, C) IHC demonstrate that CACYBP is located in both the cytoplasm and the nucleus, with higher abundance observed in the COVID-19 comparison group than that in the PQ comparison group (Scale: 50× for low magnification, 200× for high magnification; *: P



IHC analysis of CACYBP using anti-CACYBP antibody (RP1104). CACYBP was detected in a paraffin-embedded section of rat testis 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 1 ug/ml rabbit anti-CACYBP Antibody (RP1104) 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.

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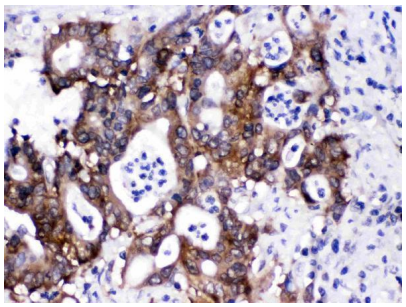
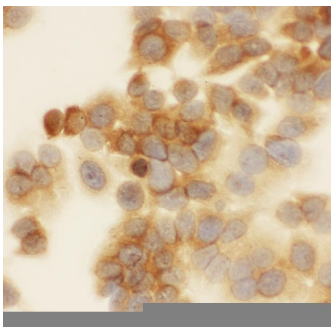
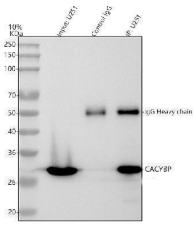


IHC analysis of CACYBP using anti-CACYBP antibody (RP1104). CACYBP was detected in a paraffin-embedded section of human intestinal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 ug/ml rabbit anti-CACYBP Antibody (RP1104) 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.



ICC analysis of CACYBP using anti-CACYBP antibody (RP1104). CACYBP was detected in immunocytochemical section of MCF-7 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-CACYBP Antibody (RP1104) 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.



Immunoprecipitating CACYBP in U251 whole cell lysate.Western blot analysis of CACYBP using anti-CACYBP antibody (RP1104). Lane 1: U251 whole cell lysates (30ug),Lane 2: Rabbit control IgG instead of anti-CACYBP antibody in U251 whole cell lysate,Lane 3: anti-CACYBP antibody (2ug) + U251 whole cell lysate (500ug).After electrophoresis, proteins were transferred to a membrane. Then the membrane was incubated with rabbit anti-CACYBP antigen affinity purified polyclonal antibody (RP1104) at a dilution of 0.5 ug/mL and probed with a goat anti-rabbit IgG-HRP secondary antibody (Catalog # BA1054). The signal is developed using ECL Plus Western Blotting Substrate (Catalog # AR1196-200). A specific band was detected for CACYBP at approximately 27 kDa. The expected band size for CACYBP is at 27 kDa.

Anti-SIAH Interacting Protein/CACYBP Antibody

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