

Anti-SIAH Interacting Protein/CACYBP Antibody

Catalog Number: PA1759

About CACYBP

CACYBP (Calcyclin-binding protein), also called SIP, is a protein that in humans is encoded by the CACYBP gene. The full-length SIP cDNA encodes a predicted 228-amino acid protein. Sequence analysis of the shortest cDNA derived by 2-hybrid screening revealed an 8-amino acid difference in the deduced open reading frame followed by a stop codon, resulting in a predicted 80-amino acid protein, SIP-short (SIPS). The CACYBP gene is mapped on 1q25.1. It may be involved in calcium-dependent ubiquitination and subsequent proteosomal degradation of target proteins. 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. The C-terminal region of SIP that is homologous to SGT1 was able to complement defects in yeast strains containing SGT1 mutant alleles, demonstrating conservation of SGT1 and SIP protein function.

Overview

Product Name	Anti-SIAH Interacting Protein/CACYBP Antibody
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-SIAH Interacting Protein/CACYBP Antibody catalog # PA1759. Tested in Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	Flow Cytometry, IF, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg Thimerosal, 0.01mg NaN3.
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 in the middle region of human CACYBP, identical to the related rat and mouse sequences.
Predicted Reactive Species	Hamster
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 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	<p>Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.</p> <p>If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.</p> <p>Some PubMed article(s) citing the expression level of this target are as follows:</p> <p>Boster Bio's internal QC testing used:</p> <p>Western blot, 0.1-0.5ug/ml, Human, Mouse, Rat</p> <p>Immunocytochemistry/Immunofluorescence, 5 ug/ml, Human</p> <p>Flow Cytometry(Fixed), 1-3 ug/1x10⁶ cells, Human</p>

Anti-SIAH Interacting Protein/CACYBP Antibody (PA1759) Images

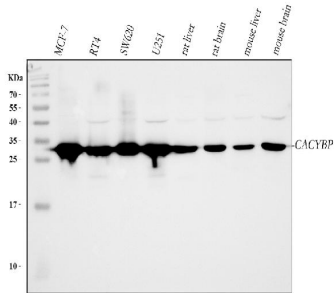


Figure 1. Western blot analysis of CACYBP using anti-CACYBP antibody (PA1759).

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 # PA1759) 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 27 kDa. The expected band size for CACYBP is at 27 kDa.

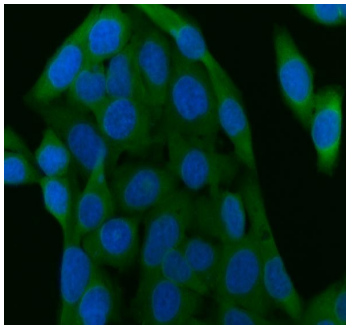


Figure 2. IF analysis of CACYBP using anti-CACYBP antibody (PA1759).

CACYBP was detected in an immunocytochemical section of Hela 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 5 ug/mL rabbit anti-CACYBP Antibody (PA1759) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:500 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.

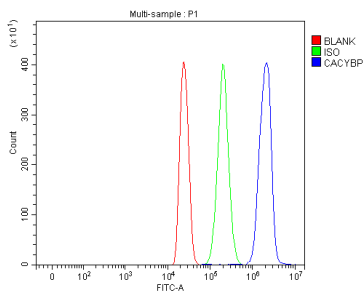


Figure 3. Flow Cytometry analysis of HL-60 cells using anti-CACYBP antibody (PA1759).

Overlay histogram showing HL-60 cells stained with PA1759 (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-CACYBP Antibody (PA1759, 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 ($1 \text{ ug}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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