Anti-CacyBP Antibody

HA500484



Product Type: Rabbit polyclonal IgG, primary antibodies

Species reactivity: Human, Mouse, Rat

Applications: WB, IF-Cell, IHC-P, FC

Molecular Wt: 26 kDa

Description: Calcyclin-binding protein is a protein that in humans is encoded by the CACYBP gene. 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. 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. CACYBP has been shown to interact with SKP1A and SIAH1.T he CacyBP/SIP complex instead, is known to be a part of

stress respons, since it interacts with chaperone HSP90.

Immunogen: Recombinant protein within human CacyBP aa 29-228 / 228.

Positive control: HepG2 cell lysate, Jurkat cell lysate, 293T cell lysate, mouse brain tissue lysate, rat spleen

tissue lysate, mouse colon tissue lysate, human colon tissue, K562, MDA-MB-468.

Subcellular location: Nucleus, Cytoplasm.

Database links: SwissProt: Q9HB71 Human | Q9CXW3 Mouse | Q6AYK6 Rat

Recommended Dilutions:

WB 1:500-1:2,000 **IF-Cell** 1:200

IHC-P 1:200-1:600 **FC** 1:500-1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at $+4^{\circ}$ C after thawing. Aliquot store at -20° C. Avoid repeated freeze / thaw cycles.

Purity: Immunogen affinity purified.

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Images

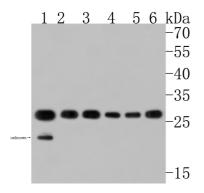


Fig1: Western blot analysis of CacyBP on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA500484, 1/1,000) was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

Positive control:

Lane 1: HepG2 cell lysate Lane 2: Jurkat cell lysate Lane 3: 293T cell lysate

Lane 4: Mouse brain tissue lysate Lane 5: Rat spleen tissue lysate Lane 6: Mouse colon tissue lysate

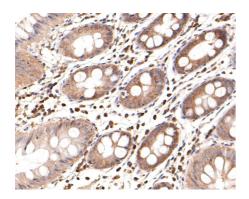


Fig2: Immunohistochemical analysis of paraffin-embedded human colon tissue using anti-CacyBP antibody. The section was pretreated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH $_2$ O and PBS, and then probed with the primary antibody (HA500484, 1/600) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

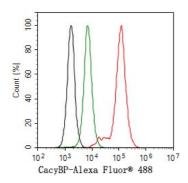


Fig3: Flow cytometric analysis of K562 cells labeling CacyBP.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA500484, 1ug/ml) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4 $^{\circ}$ C for an hour, the cells were stained with a Alexa Fluor® 488 conjugate-Goat anti-Rabbit IgG Secondary antibody at 1/1,000 dilution for 30 minutes at +4 $^{\circ}$ C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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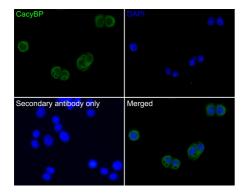


Fig4: Immunocytochemistry analysis of MDA-MB-468 cells labeling CacyBP with Rabbit anti-CacyBP antibody (HA500484) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 $^{\circ}$ C, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-CacyBP antibody (HA500484) at 1/200 dilution in 2% negative goat serum overnight at 4 $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor **M** 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

- 1. Bohush A. et. al. HSP90 Co-Chaperone, CacyBP/SIP, Protects alpha-Synuclein from Aggregation. Cells. 2020 Oct
- 2. Jiang TX. et. al. SIP/CacyBP promotes autophagy by regulating levels of BRUCE/Apollon, which stimulates LC3-I degradation. Proc Natl Acad Sci U S A. 2019 Jul