

Anti-N4BP1 Antibody Picoband®

Catalog Number: A12575-3

About N4BP1

Nedd4-binding partner-1 (N4BP1) has been identified as a protein interactor and a substrate of the homologous to E6AP C terminus (HECT) domain-containing E3 ubiquitin-protein ligase (E3), Nedd4. Here, we describe a previously unrecognized functional interaction between N4BP1 and Itch, a Nedd4 structurally related E3, which contains four WW domains, conferring substrate-binding activity. We show that N4BP1 association with the second WW domain (WW2) of Itch interferes with E3 binding to its substrates. In particular, we found that N4BP1 and p73alpha, a target of Itch-mediated ubiquitin/proteasome proteolysis, share the same binding site. By competing with p73alpha for binding to the WW2 domain, N4BP1 reduces the ability of Itch to recruit and ubiquitylate p73alpha and inhibits Itch autoubiquitylation activity both in vitro and in vivo ubiquitylation assays.

Overview

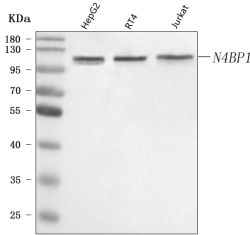
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| Product Name | Anti-N4BP1 Antibody Picoband® |
| Reactive Species | Human |
| Description | Boster Bio Anti-N4BP1 Antibody Picoband® catalog # A12575-3. Tested in ELISA, IF, ICC, WB, Flow Cytometry applications. This antibody reacts with Human. The 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 | ELISA, Flow Cytometry, IF, ICC, WB |
| Clonality | Polyclonal |
| Formulation | Each vial contains 4 mg Trehalose, 0.9 mg NaCl, 0.2 mg Na ₂ HPO ₄ . |
| Storage Instructions | 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 freezing and thawing. |
| Host | Rabbit |
| Uniprot ID | O75113 |

Technical Details

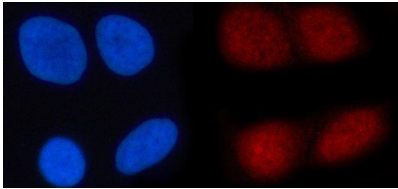
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|-------------------------------|---|
| Immunogen | E.coli-derived human N4BP1 recombinant protein (Position: M1-K885). |
| Recommended Detection Systems | Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, 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 |

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| Form | Lyophilized |
| Concentration | Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml. |
| Purification | Immunogen affinity purified. |
| Suggested Dilutions | Western blot, 0.25-0.5 µg/ml, Human Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human Flow Cytometry (Fixed), 1-3 µg/1x10 ⁶ cells, Human ELISA, 0.1-0.5 µg/ml, - |

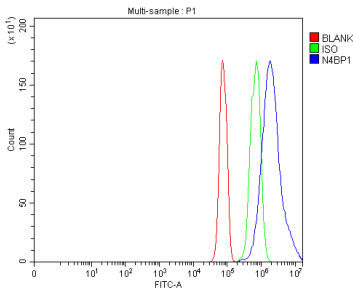
Anti-N4BP1 Antibody Picoband® (A12575-3) Images



Western blot analysis of N4BP1 using anti-N4BP1 antibody (A12575-3). 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 RT4 whole cell lysates, Lane 3: human Jurkat whole cell 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-N4BP1 antigen affinity purified polyclonal antibody (Catalog # A12575-3) 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 N4BP1 at approximately 110 kDa. The expected band size for N4BP1 is at 100 kDa.



IF analysis of N4BP1 using anti-N4BP1 antibody (A12575-3). N4BP1 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-N4BP1 Antibody (A12575-3) overnight at 4°C. Cy3 Conjugated Goat Anti-Rabbit IgG (BA1032) 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.



Flow Cytometry analysis of JK cells using anti-N4BP1 antibody (A12575-3). Overlay histogram showing JK cells stained with A12575-3 (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-N4BP1 Antibody (A12575-3, 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.

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Anti-N4BP1 Antibody

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