BOSTER BIOLOGICAL TECHNOLOGY
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Anti-NEDD4 Antibody Picoband®

Catalog Number: A00984-3

About NEDD4

E3 ubiquitin-protein ligase NEDD4, also known as neural precursor cell expressed developmentally down-regulated protein 4 (NEDD4), is an enzyme that in humans is encoded by the NEDD4 gene. This gene is the founding member of the NEDD4 family of HECT ubiquitin ligases that function in the ubiquitin proteasome system of protein degradation. The encoded protein contains an N-terminal calcium and phospholipid binding C2 domain followed by multiple tryptophan-rich WW domains and, a C-terminal HECT ubiquitin ligase catalytic domain. It plays critical role in the regulation of a number of membrane receptors, endocytic machinery components and the tumor suppressor PTEN.

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Overview

Product Name	Anti-NEDD4 Antibody Picoband®
Reactive Species	Human, Rat
Description	Boster Bio Anti-NEDD4 Antibody Picoband® catalog # A00984-3. Tested in ELISA, Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, 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	ELISA, Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl, 0.2 mg Na2HPO4.
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	P46934

Technical Details

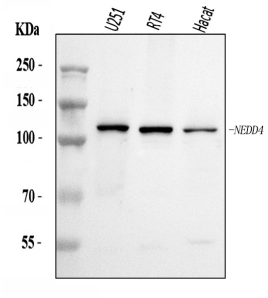
Immunogen	E.coli-derived human NEDD4 recombinant protein (Position: M1-A736).
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western

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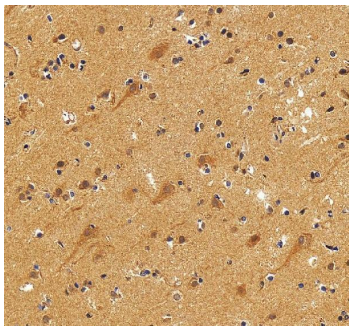
	blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.1-0.25 ug/ml, Human Immunohistochemistry(Paraffin-embedded Section), 2-5 ug/ml, Human, Rat Immunocytochemistry/Immunofluorescence, 5 ug/ml, Human Flow Cytometry (Fixed), 1-3 ug/1x10^6 cells, Human ELISA, 0.1-0.5 ug/ml, - Note: For flow cytometry (FCM), suitable for fixed cells only. Not suitable for FACS or surface flow cytometry using live cells.

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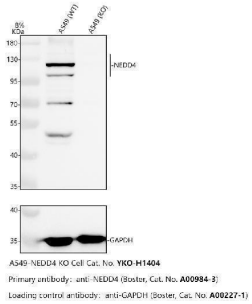
Anti-NEDD4 Antibody Picoband® (A00984-3) Images



Western blot analysis of NEDD4 using anti-NEDD4 antibody (A00984-3). 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 U251 whole cell lysates, Lane 2: human RT4 whole cell lysates, Lane 3: human Hacat 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-NEDD4 antigen affinity purified polyclonal antibody (Catalog # A00984-3) at 0.25 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 NEDD4 at approximately 115 kDa. The expected band size for NEDD4 is at 115 kDa.



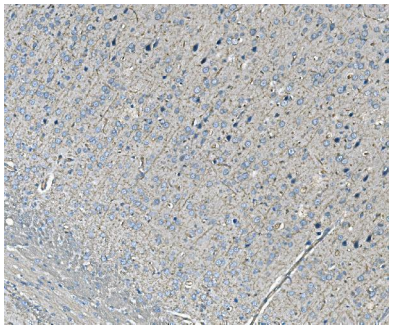
IHC analysis of NEDD4 using anti-NEDD4 antibody (A00984-3). NEDD4 was detected in a paraffin-embedded section of human brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-NEDD4 Antibody (A00984-3) 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.



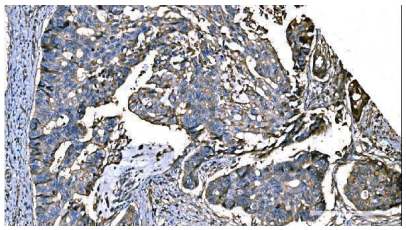
Western blot analysis of NEDD4 using anti-NEDD4 antibody (A00984-3). Electrophoresis was performed on a 8% SDS-PAGE gel at 80V (Stacking gel) / 120V (Resolving gel) for 2 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human A549-WT whole cell lysates, Lane 2: human A549-NEDD4 KO 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-NEDD4 antigen affinity purified polyclonal antibody (A00984-3) 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 ECL Plus Western Blotting Substrate (Catalog # AR1196-200) with Tanon 5200 system. A specific band was detected for NEDD4 at approximately 105-150 kDa. The expected band size for NEDD4 is at 149

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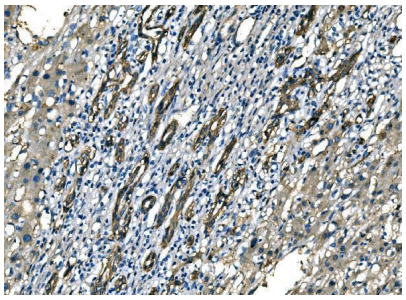
kDa.



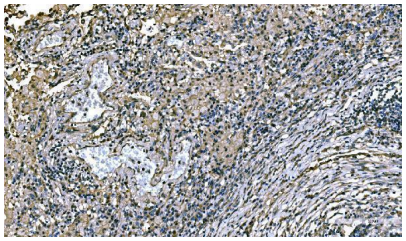
IHC analysis of NEDD4 using anti-NEDD4 antibody (A00984-3). NEDD4 was detected in a paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-NEDD4 Antibody (A00984-3) 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 NEDD4 using anti-NEDD4 antibody (A00984-3). NEDD4 was detected in a paraffin-embedded section of human colon adenocarcinoma 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-NEDD4 Antibody (A00984-3) 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.

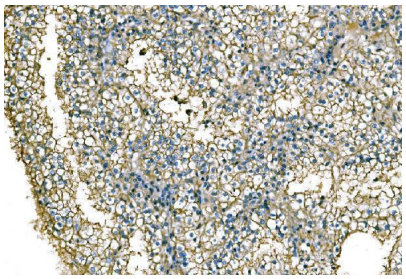


IHC analysis of NEDD4 using anti-NEDD4 antibody (A00984-3). NEDD4 was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-NEDD4 Antibody (A00984-3) 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.

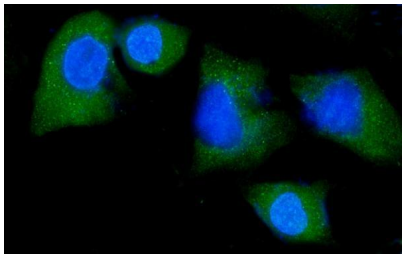


IHC analysis of NEDD4 using anti-NEDD4 antibody (A00984-3). NEDD4 was detected in a paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-NEDD4 Antibody (A00984-3) 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.

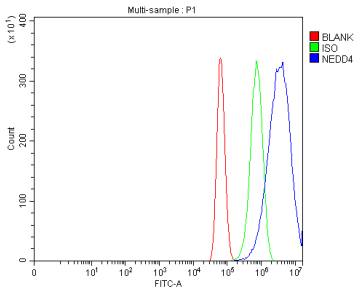
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IHC analysis of NEDD4 using anti-NEDD4 antibody (A00984-3). NEDD4 was detected in a paraffin-embedded section of human renal cell carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-NEDD4 Antibody (A00984-3) 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.



IF analysis of NEDD4 using anti-NEDD4 antibody (A00984-3). NEDD4 was detected in an immunocytochemical section of A549 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-NEDD4 Antibody (A00984-3) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 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 U87 cells using anti-NEDD4 antibody (A00984-3). Overlay histogram showing U87 cells stained with A00984-3 (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-NEDD4 Antibody (A00984-3, 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 without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

Anti-NEDD4 Antibody

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