BOSTER BIOLOGICAL TECHNOLOGY
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Anti-HEF1/NEDD9 Antibody Picoband®

Catalog Number: PB9289

About NEDD9

Enhancer of filamentation 1 (EF1), also known as NEDD-9, is a protein that in humans is encoded by the NEDD9 gene. The protein encoded by this gene is a member of the CRK-associated substrates family. Members of this family are adhesion docking molecules that mediate protein-protein interactions for signal transduction pathways. It is mapped to 6p24.2. This protein is a focal adhesion protein that acts as a scaffold to regulate signaling complexes important in cell attachment, migration and invasion as well as apoptosis and the cell cycle. This protein has also been reported to have a role in cancer metastasis. NEDD9 is also a highly relevant cancer gene that governs metastatic potential in human melanoma. What's more, it has been speculated that NEDD9 may be an important linking element between extracellular signaling and regulation of the cytoskeleton.

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Overview

Product Name	Anti-HEF1/NEDD9 Antibody Picoband®
Reactive Species	Human, Monkey, Mouse, Rat
Description	Boster Bio Anti-HEF1/NEDD9 Antibody Picoband® catalog # PB9289. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Monkey, 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, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.
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	Q14511

Technical Details

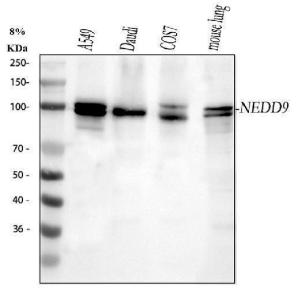
Immunogen	E.coli-derived human HEF1 recombinant protein (Position: K273-E421). Human HEF1 shares 83% amino acid (aa) sequence identity with mouse HEF1.

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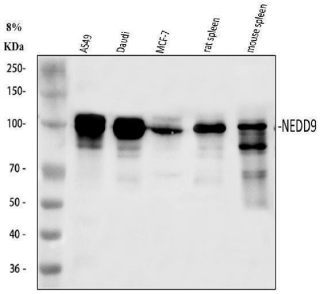
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG
Form	Lyophilized
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, Monkey, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human Immunocytochemistry/Immunofluorescence, 5ug/ml, Human Flow Cytometry (Fixed), 1-3ug/1x10^6 cells, Human 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-HEF1/NEDD9 Antibody Picoband® (PB9289) Images



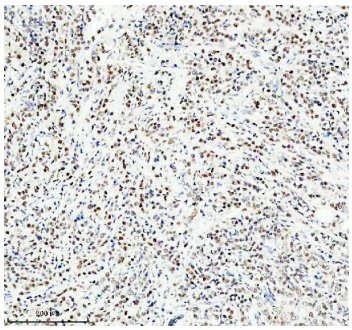
Western blot analysis of HEF1 using anti-HEF1 antibody (PB9289). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human A549 whole cell lysates, Lane 2: human Daudi whole cell lysates, Lane 3: monkey COS-7 whole cell lysates, Lane 4: mouse lung 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-HEF1 antigen affinity purified polyclonal antibody (Catalog # PB9289) 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 HEF1 at approximately 93-105 kDa. The expected band size for HEF1 is at 93 kDa.



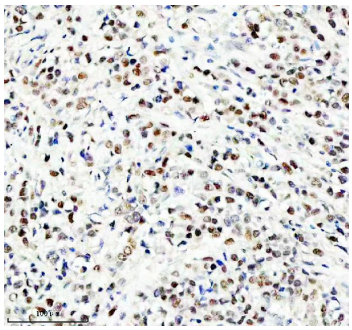
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IHC analysis of HEF1 using anti-HEF1 antibody (PB9289). HEF1 was detected in a paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-HEF1 Antibody (PB9289) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C.

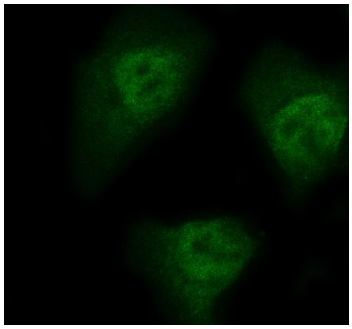
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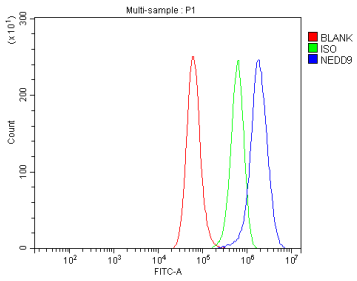
The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.



IHC analysis of HEF1 using anti-HEF1 antibody (PB9289). HEF1 was detected in a paraffin-embedded section of human breast 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-HEF1 Antibody (PB9289) 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 HEF1 using anti-HEF1 antibody (PB9289). HEF1 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-HEF1 Antibody (PB9289) 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. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



Flow Cytometry analysis of A549 cells using anti-HEF1 antibody (PB9289). Overlay histogram showing A549 cells stained with PB9289 (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-HEF1 Antibody (PB9289, 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.

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