

Product Datasheet

HEF1/NEDD9 Rabbit Polyclonal Antibody (orb234335)

Catalog Number	orb234335
Category	Antibodies
Description	Anti-HEF1/NEDD9 Antibody. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Monkey, Mouse, Rat.
Target	Enhancer of filamentation 1
Clonality	Polyclonal
Species/Host	Rabbit
Isotype	Rabbit IgG
Conjugation	Unconjugated
Reactivity	Human, Monkey, Mouse, Rat
Form/Appearance	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
Buffer/Preservatives	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na ₂ HPO ₄ .
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.
Purification	Immunogen affinity purified.
Immunogen	E.coli-derived human HEF1 recombinant protein (Position: K273-E421). Human HEF1 shares 83% amino acid (aa) sequence identity with mouse HEF1.
UniProt ID	Q14511

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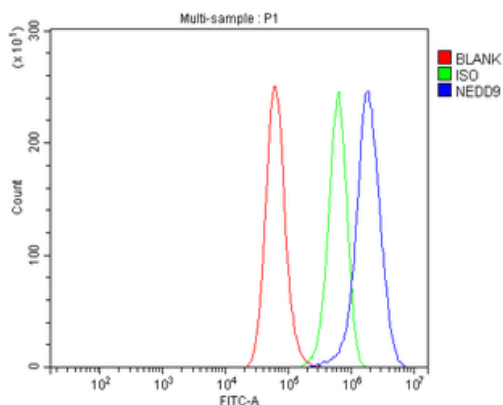
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MW	93-105 kDa
Tested applications	FC, ICC, IF, IHC, WB
Dilution range	Western blot, 0.1-0.5µg/ml, Human, Monkey, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5µg/ml, Human Immunocytochemistry/Immunofluorescence, 5µg/ml, Human Flow Cytometry (Fixed), 1-3µg/1x10 ⁶ cells, Human
Specificity	No cross reactivity with other proteins.
Cross Reactivity	No cross-reactivity with other proteins
Antibody Type	Primary Antibody
Storage	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
Note	For research use only
Expiration Date	12 months from date of receipt.



Flow Cytometry analysis of A549 cells using anti-HEF1 antibody. Overlay histogram showing A549 cells (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 (1 µg/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 µg/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 µg/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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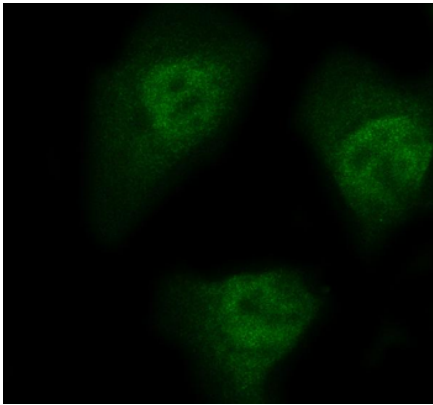
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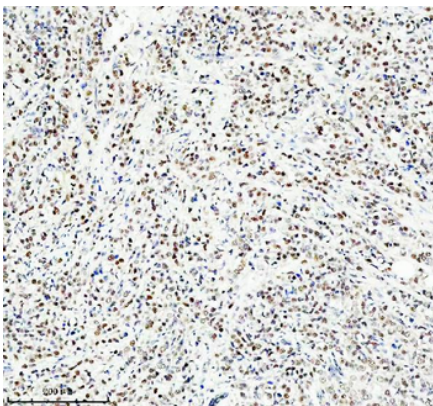
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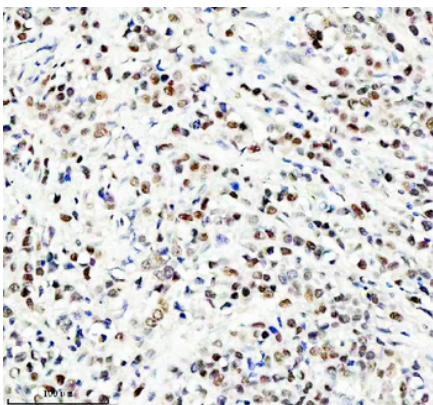
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IF analysis of HEF1 using anti-HEF1 antibody. HEF1 was detected in an immunocytochemical section of HeLa cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL rabbit anti-HEF1 Antibody overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG 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.



IHC analysis of HEF1 using anti-HEF1 antibody. HEF1 was detected in a paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-HEF1 Antibody 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 with DAB as the chromogen.



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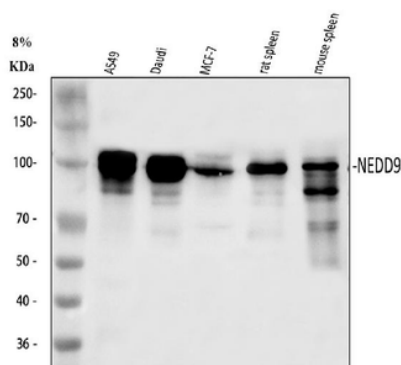
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Western blot analysis of HEF1 using anti-HEF1 antibody. Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human A549 whole cell lysates, Lane 2: human Daudi whole cell lysates, Lane 3: human MCF-7 whole cell lysates, Lane 4: rat spleen tissue lysates, Lane 5: mouse spleen 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 at 0.5 µg/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 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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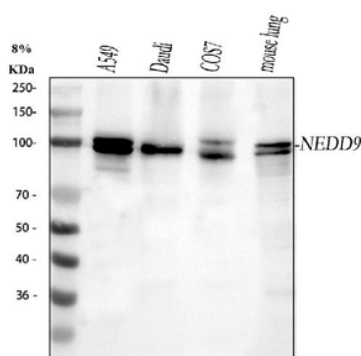
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