

NEDD8 Polyclonal Antibody

Catalog NumberPA5-17476

Product data sheet

| Details | | Species Reactivity | |
|--------------------|---|--|--------------------------------------|
| Size | 100 µL | Species reactivity | Human, Mouse, Non-human primate, Rat |
| Host/Isotope | Rabbit / IgG | Published species | Human, Mouse |
| Class | Polyclonal | Tested Applications | |
| Type | Antibody | Dilution * | |
| Immunogen | Synthetic peptide corresponding to amino acids at the amino-terminus of human NEDD8 protein | Immunohistochemistry (Paraffin) (IHC (P)) | 1:200 |
| Conjugate | Unconjugated | Immunoprecipitation (IP) | 1:25 |
| Form | Liquid | Western Blot (WB) | 1:1,000 |
| Concentration | 162 µg/mL | Immunocytochemistry (ICC/IF) | 1:100 |
| Purification | Antigen affinity chromatography | Published Applications | |
| Storage buffer | 0.01M HEPES, pH 7.5, with 0.15M NaCl, 100µg/mL BSA, 50% glycerol | Western Blot (WB) | See 1 publications below |
| Contains | no preservative | * Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls. | |
| Storage Conditions | -20°C | | |

Product specific information

It is not recommended to aliquot this antibody. This antibody is not cross-reactive with other ubiquitin family members, including ubiquitin, SUMO1, SUMO2, SUMO3 and ISG15.

Background/Target Information

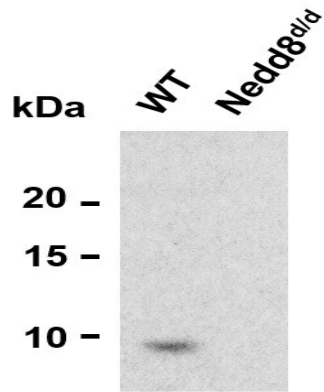
NEDD8 is a ubiquitin-like protein which plays an important role in cell cycle control and embryogenesis. Covalent attachment to its substrates requires prior activation by the E1 complex UBE1C-APPBP1 and linkage to the E2 enzyme UBE2M. Attachment of NEDD8 to cullins activates their associated E3 ubiquitin ligase activity, and thus promotes polyubiquitination and proteasomal degradation of cyclins and other regulatory proteins.

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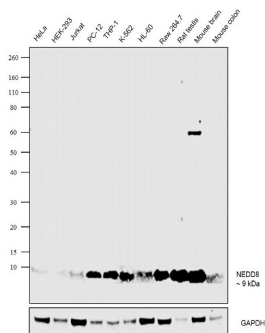


NEDD8 Antibody (PA5-17476)

Antibody specificity was demonstrated by CRISPR-Cas9 mediated knockout of target protein Nedd8. A loss of signal by western blot was observed for targeted Nedd8 KO cell line (lane 2) compared to gRNA cell line (lane 1) using polyclonal Nedd8 antibody (Product # PA5-17476). {KO}

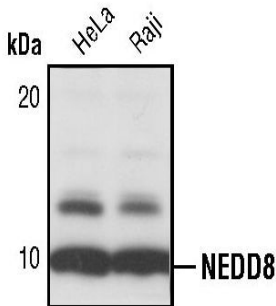
NEDD8 Antibody (PA5-17476) in WB

Western blot was performed using anti-NEDD8 Polyclonal Antibody (Product # PA5-17476) and a 9 kDa band corresponding to NEDD8 was observed across all the cell lines tested. Whole cell extracts (30 µg lysate) of HeLa (Lane 1), HEK-293 (Lane 2), Jurkat (Lane 3), PC-12 (Lane 4), THP-1 (Lane 5), K-562 (Lane 6), HL-60 (Lane 7), Raw 264.7 (Lane 8), tissue extracts of Rat testis (Lane 9), Mouse brain (Lane 10) and Mouse colon (Lane 11) were electrophoresed Novex® NuPAGE® 12 % Bis-Tris gel (Product # NP0342BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with the primary antibody (1:1000 dilution) and detected by chemiluminescence with Goat anti-Rabbit IgG (Heavy Chain), Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:4000 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005).



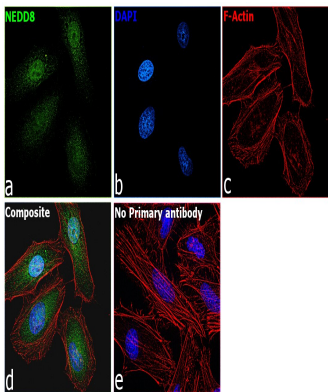
NEDD8 Antibody (PA5-17476) in WB

Western blot analysis of NEDD8 in lysates from HeLa and Raji cells using NEDD8 polyclonal antibody (Product # PA5-17476).



NEDD8 Antibody (PA5-17476) in ICC/IF

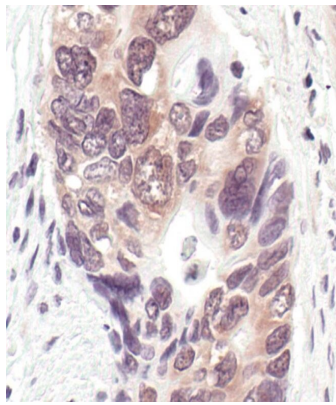
Immunofluorescence analysis of NEDD8 was performed using 70% confluent log phase HeLa cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, and blocked with 2% BSA for 1 hour at room temperature. The cells were labeled with NEDD8 Polyclonal Antibody (Product # PA5-17476) at 1:100 dilution in 0.1% BSA, incubated at 4 degree Celsius overnight and then labeled with Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A27034) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing localization to the cytoplasm and nucleus. Panel e represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.



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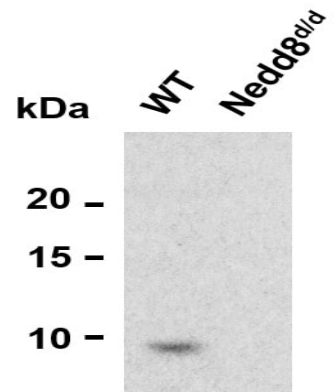
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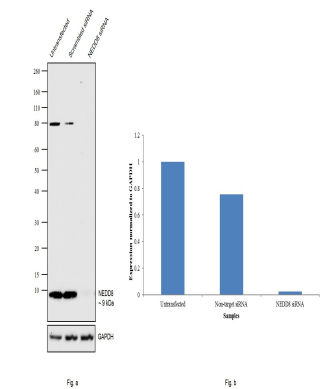
NEDD8 Antibody (PA5-17476) in IHC (P)

Immunohistochemical analysis of NEDD8 in paraffin-embedded human colon carcinoma using a NEDD8 polyclonal antibody (Product # PA5-17476) showing cytoplasmic and membrane localization.



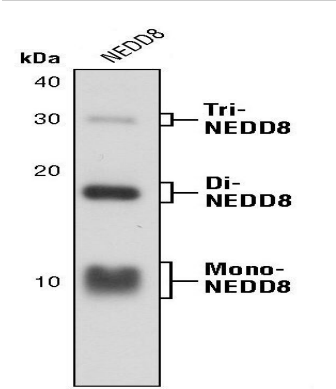
NEDD8 Antibody (PA5-17476) in WB

Western blot analysis of Nedd8 was performed by loading whole cell lysate in 1X LDS sample buffer with 2-ME from 5 x 10⁵ mouse transformed pre-B cells. Samples were loaded onto a 4-12 % Bis-Tris polyacrylamide gel (Product # NP0336BOX). Proteins were transferred to nitrocellulose membrane with wet/tank transfer. Membrane was blocked in 5% milk/TBST. The membrane was probed with a polyclonal anti-Nedd8 antibody (Product # PA5-17476) at a dilution of 1:500 in 5% milk/TBST at 4°C overnight, followed by a secondary antibody HRP-anti-rabbit at a dilution of 1:10000 at room temperature for 1 hour. Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate (Product # PI-32209). Data courtesy of Thermo Scientific KOL Program.



NEDD8 Antibody (PA5-17476) in WB

Knockdown of NEDD8 was achieved by transfecting HeLa with NEDD8 specific siRNAs (Silencer® select Product # s226684, s9424). Western blot analysis (Fig. a) was performed using whole cell extracts from the NEDD8 knockdown cells (lane 3), non-specific scrambled siRNA transfected cells (lane 2) and untransfected cells (lane 1). The blot was probed with NEDD8 Polyclonal Antibody (Product # MA5-17476, 1:1000 dilution) and Goat anti-Rabbit IgG (Heavy Chain), Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:4000 dilution). Densitometric analysis of this western blot is shown in histogram (Fig. b). Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to NEDD8.



NEDD8 Antibody (PA5-17476) in WB

Western blot analysis of NEDD8 recombinant protein (5 ng) using NEDD8 polyclonal antibody (Product # PA5-17476).

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PubMed References For NEDD8 Polyclonal Antibody

1 Western Blot References

| Species / Dilution | Summary |
|--------------------|--|
| Mouse / 1:1000 | <p>PA517476 was used in western blot to test if increased NUB1 protein levels reduces mutant huntingtin levels through a proteasome dependent mechanism</p> <p>Journal of Huntington's disease (2016; 5: 163)</p> <p>"Effects of Exogenous NUB1 Expression in the Striatum of HDQ175/Q7 Mice."</p> <p>Author(s):Vodicka P,Chase K,Iuliano M,Valentine DT,Sapp E,Lu B,Kegel-Gleason KB,Sena-Esteves M,Aronin N,DiFiglia M</p> <p>PubMed Article URL:http://dx.doi.org/10.3233/JHD-160195</p> |

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