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antibody and ELISA experts

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# Anti-NEDD8 Antibody Picoband™

**Catalog Number:** A00547

## About NEDD8

NEDD8 is a protein that in humans is encoded by the NEDD8 gene. Human NEDD8 shares 60% amino acid sequence identity to ubiquitin. The most substrates of NEDD8 modification are the Cullin subunits of Cullin-based E3 ubiquitin ligases, which are active only when neddylated. Their NEDDylation is critical for the recruitment of E2 to the ligase complex, thus facilitating ubiquitin conjugation. NEDD8 modification has therefore been implicated in cell cycle progression and cytoskeletal regulation.

## Overview

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| Product Name | Anti-NEDD8 Antibody Picoband™ |
| Reactive Species | Human, Mouse, Rat |
| Description | Boster Bio Anti-NEDD8 Antibody Picoband™ catalog # A00547. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat. |
| Application | Flow Cytometry, IF, IHC, ICC, WB |
| Clonality | Polyclonal |
| Formulation | Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg $Na_2HPO_4$, 0.05mg $NaN_3$. |
| 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 | Q15843 |

## Technical Details

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| Immunogen | A synthetic peptide corresponding to a sequence in the middle region of human NEDD8, identical to the related mouse and rat sequences. |
| Predicted Reactive Species | Chicken |
| 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. |

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| Purification | Immunogen affinity purified. |
| Suggested Dilutions | Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.<br>If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.<br>Some PubMed article(s) citing the expression level of this target are as follows:<br>Boster Bio's internal QC testing used:<br>Western blot, 0.1-0.5ug/ml, Human, Mouse, Rat<br>Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, By Heat<br>Immunocytochemistry/Immunofluorescence, 2ug/ml, Human<br>Flow Cytometry, 1-3ug/1x$10^6$ cells, Human |

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# Anti-NEDD8 Antibody Picoband™ (A00547) Images

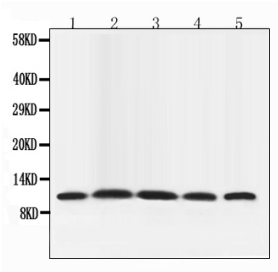


Figure 1. Western blot analysis of NEDD8 using anti-NEDD8 antibody (A00547).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: rat testis cell lysates,
Lane 2: mouse thymus tissue lysates,
Lane 3: mouse brain tissue lysates,
Lane 4: HELA whole cell lysates,
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA 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-NEDD8 antigen affinity purified polyclonal antibody (Catalog # A00547) 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 1:10000 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 NEDD8 at approximately 12KD. The expected band size for NEDD8 is at 9KD.

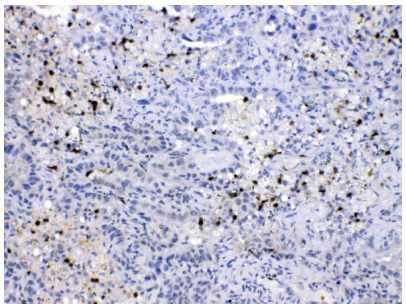

Figure 2. IHC analysis of NEDD8 using anti-NEDD8 antibody (A00547).
NEDD8 was detected in paraffin-embedded section of human lung cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-NEDD8 Antibody (A00547) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

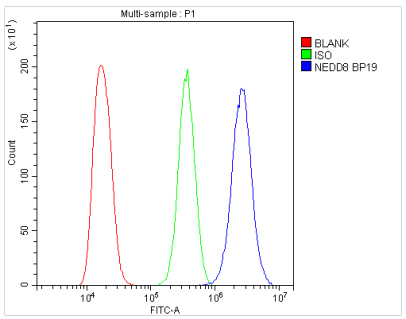


Figure 3. Flow Cytometry analysis of A431 cells using anti-NEDD8 antibody (A00547).
Overlay histogram showing A431 cells stained with A00547 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-NEDD8 Antibody (A00547,1ug/1x$10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x$10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x$10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Figure 4. IF analysis of NEDD8 using anti-NEDD8 antibody (A00547).
NEDD8 was detected in immunocytochemical section of

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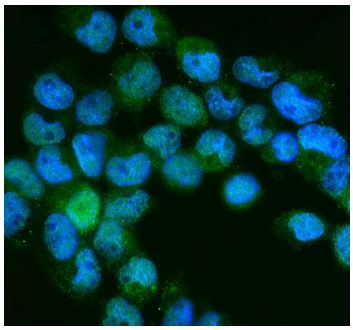

A431 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2ug/mL rabbit anti-NEDD8 Antibody (A00547) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was 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.

## 1 Publications Citing This Product

1. PubMed ID: 32456285, Jiang B,Adams Z,Moonah S,Shi H,Maupin-Furlow J,Moskovitz J.The Antioxidant Enzyme Methionine Sulfoxide Reductase A (MsrA) Interacts with Jab1/CSN5 and Regulates Its Function.Antioxidants (Basel).2020 May 24;9(5):452.doi:10.3390/antiox9050452.PMID:32456285

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