abcam

## ab81264

# Anti-NEDD8 antibody [Y297]

Anti-NEDD8 antibody [Y297] (ab81264) is a rabbit monoclonal antibody detecting NEDD8 in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF**. Suitable for **Human, Mouse, Rat**.

- Biophysical QC for unrivalled batch-batch consistency
- Over 40 publications
- Trusted since 2009

Recombinant | RabMAb | 20ul selling size

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA |
| **Form** | Liquid |
| **Clonality** | Monoclonal |
| **Immunogen** | The exact immunogen used to generate this antibody is proprietary information. |
| **Clone number** | Y297 |
| **Purification technique** | Affinity purification Protein A |
| **Specificity** | This antibody detects NEDD8 bound to cullins proteins (neddylated cullins) as well as free NEDD8. |
| **Concentration** | 0.261 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

### IP

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/20 - 1/50 |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Rat, Mouse |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

## WB

### Tested

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | 1/1000 - 1/10000 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/1000 - 1/10000 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/1000 - 1/10000 |
| **Notes** | - |

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/100 - 1/250 |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Rat, Mouse |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

## Flow Cyt (Intra)

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/300 |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Rat, Mouse |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

## IHC-P

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/100 - 1/500 |
| **Notes** | Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |

### Expected

| | |
|---|---|
| **Species** | Rat, Mouse |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

# Target data

See full target information NEDD8

| | |
|---|---|
| **Function** | Ubiquitin-like protein which plays an important role in cell cycle control and embryogenesis via its conjugation to a limited number of cellular proteins, such as cullins or p53/TP53 (PubMed:10318914, PubMed:10597293, PubMed:11953428, PubMed:14690597, PubMed:15242646, PubMed:9694792, PubMed:38605244, PubMed:38316879). Attachment of NEDD8 to cullins is critical for the recruitment of E2 to the cullin-RING-based E3 ubiquitin-protein ligase complex, thus facilitating polyubiquitination and proteasomal degradation of cyclins and other regulatory proteins (PubMed:10318914, PubMed:10597293, PubMed:11953428, PubMed:20688984, PubMed:9694792, PubMed:38605244, PubMed:38316879). Attachment of NEDD8 to p53/TP53 inhibits p53/TP53 transcriptional activity (PubMed:15242646). Covalent attachment to its substrates requires prior activation by the E1 complex UBE1C-APPBP1 and linkage to the E2 enzyme UBE2M (PubMed:14690597). |

## Storage

| | |
|---|---|
| **Shipped at conditions** | Conditional Ambient |
| **Appropriate short-term storage duration** | 1-2 weeks |
| **Appropriate short-term storage conditions** | +4°C |
| **Appropriate long-term storage conditions** | -20°C |
| **Aliquoting information** | Upon delivery aliquot |
| **Storage information** | Stable for 12 months at -20°C |

## Notes

**What is this antibody validated in?**
Anti-NEDD8 antibody [Y297] (ab81264) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human, Mouse, Rat samples.

**What is the molecular weight of NEDD8?**
Anti-NEDD8 [Y297] (ab81264) specifically detects a band for NEDD8 (UniProt: Q15843) at a molecular weight of 9kDa.

**Trusted by the scientific community**
Anti-NEDD8 [Y297] (ab81264) was first used in a scientific publication in 2009 and has been cited over 40 times in peer-reviewed journals.

**Other related products**
We have a range of other formats of antibody clone [Y297] also available for your convenience: ab81264, Alexa Fluor® 647 - ab200849, Alexa Fluor® 488 - ab201249, Alexa Fluor® 594 - ab207247, Alexa Fluor® 405 - ab207248,

Alexa Fluor® 555 - ab207249, Carrier free - ab220816, PE - ab305725, APC - ab305726, HRP - ab305727, Alkaline Phosphatase - ab308828, Alexa Fluor® 568 - ab312568, Alexa Fluor® 750 - ab321737

**Patented technology**
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

**What are the advantages of a recombinant monoclonal antibody?**
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

**Trial sizes available!**
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

## Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

| | |
|---|---|
| **Activity summary** | NEDD8 also known as neural precursor cell expressed developmentally downregulated protein 8 is a small ubiquitin-like protein with a molecular weight of approximately 9 kDa. It is expressed in various tissues prominently in the brain and muscles. NEDD8 conjugates to target proteins modifying their functions by altering their stability activity or location. It plays a role in the regulation of protein degradation. |
| **Biological function summary** | The NEDD8 protein is part of a modification system that activates cullin-RING ligase complexes. This activation promotes the ubiquitination and subsequent proteasomal degradation of targeted proteins. As a post-translational modification system similar to ubiquitin NEDD8 is important for maintaining protein homeostasis by ensuring timely degradation and turnover of cellular proteins. These processes impact cell cycle progression signal transduction and stress responses. |
| **Pathways** | NEDD8 is a central player in the ubiquitin-proteasome system an essential pathway for protein turnover. It is particularly important in the NEDDylation pathway where it regulates protein functions by transiently attaching to substrate proteins. Through these processes NEDD8 interacts closely with proteins like cullins and ubiquitin affecting cellular responses and maintaining cellular integrity under different conditions. |
| **Associated diseases and disorders** | NEDD8 influences several pathological states. Aberrant NEDD8 conjugation can lead to cancer by disrupting the controlled degradation of proteins involved in cell cycle regulation such as p27 and p21. Additionally improper NEDDylation has links to neurodegenerative diseases like Alzheimer's as it affects proteins related to |

synaptic function and neuronal survival. Understanding the role of NEDD8 in these diseases aids in the development of potential therapeutic strategies.

## Product promise

**Tested**

We have tested this species and application combination and it works. It is covered by our product promise.

**Expected**

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

**Predicted**

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

**Not recommended**

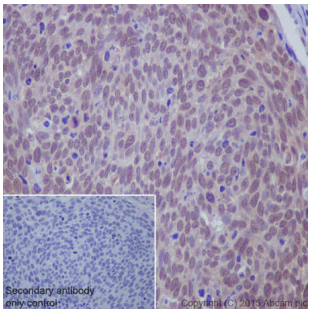
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

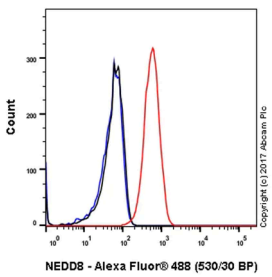
Full details and terms and conditions can be found here:
Terms & Conditions.

## 11 product images




### Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NEDD8 antibody [Y297] (ab81264)

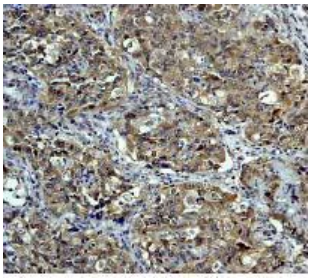
Immunohistochemical staining of paraffin embedded human cervical carcinoma with purified ab81264 at a working dilution of 1/400. The secondary antibody used is ab97051, a goat anti-rabbit IgG (H&L) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.




## Flow Cytometry (Intracellular) - Anti-NEDD8 antibody [Y297] (ab81264)

NEDD8 Flow Cytometry (Intracellular) staining using rabbit Anti-NEDD8 antibody

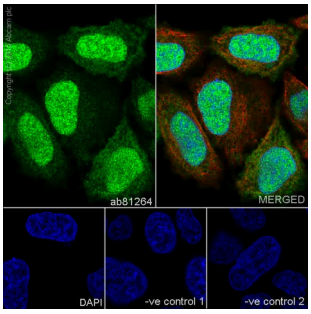
Intracellular Flow Cytometry analysis of A-673 (Human muscle Ewing's Sarcoma) cells labeling NEDD8 (red) with purified ab81264 at a 1/300 dilution (1ug/mL). Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti rabbit IgG (Alexa Fluorr® 488) (ab150077) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal IgG (Black) (ab172730). Blue (unlabeled control) - Cell without incubation with primary antibody and secondary antibody (Blue).



## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NEDD8 antibody [Y297] (ab81264)

NEDD8 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-NEDD8 antibody

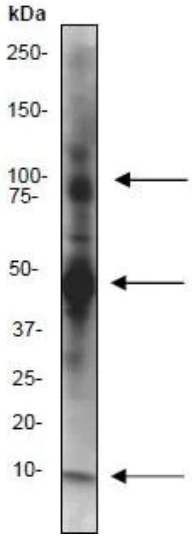
Unpurified ab81264, at 1/100 dilution, staining NEDD8 in human lung carcinoma tissue by Immunohistochemistry using formalin-fixed, paraffin-embedded section.




## Immunocytochemistry/ Immunofluorescence - Anti-NEDD8 antibody [Y297] (ab81264)

NEDD8 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-NEDD8 antibody

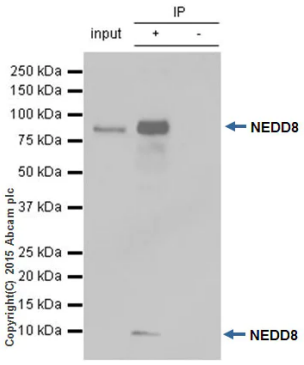
Immunofluorescence staining of HeLa cells with purified ab81264 at a working dilution of 1/100, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (ab150077), used at a dilution of 1/1000. ab7291, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with ab150120 (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab81264 was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (ab150120) at a dilution of 1/500. For negative control 2, ab7291 (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (ab150077) at a dilution of 1/400.




## Immunoprecipitation - Anti-NEDD8 antibody [Y297] (ab81264)

Western blot analysis of immunoprecipitation pellet from HeLa cell lysate using unpurified ab81264 at 1/500 dilution. Band at 90 kDa shows Neddylated cullins, 50 kDa the IgG fraaction and 9 kDa Free NEDD8.
All lanes:
Immunoprecipitation - Anti-NEDD8 antibody [Y297] (ab81264)

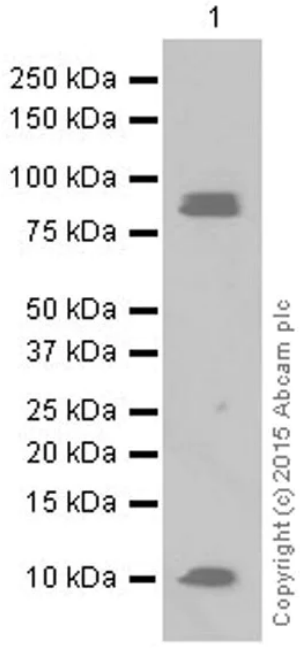
Predicted band size: 9 kDa




## Immunoprecipitation - Anti-NEDD8 antibody [Y297] (ab81264)

ab81264 (purified) at 1/20 immunoprecipitating NEDD8 in 10 µg K562 cell lysate (Lanes 1 and 2, observed at 10 and 90 kDa). Lane 3 - Rabbit monoclonal IgG (ab172730). For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10,000 dilution. Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST
All lanes:
Immunoprecipitation - Anti-NEDD8 antibody [Y297] (ab81264)

Predicted band size: 9 kDa




## Western blot - Anti-NEDD8 antibody [Y297] (ab81264)

NEDD8 Western blot staining of K562 whole cell lysate using rabbit Anti-NEDD8 antibody

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST
The band at 12kDa is free NEDD8, and at 90kDa is Neddylated cullins.

All lanes:
Western blot - Anti-NEDD8 antibody [Y297] (ab81264) at 1/1000 dilution

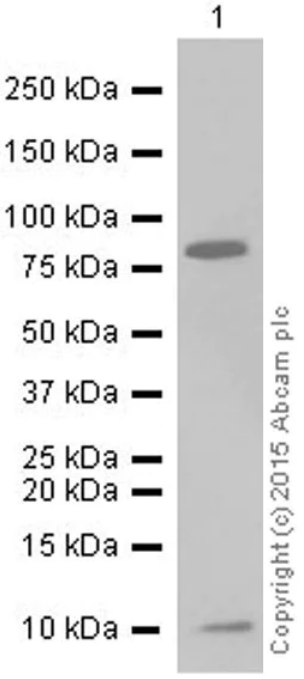
All lanes:
K562 whole cell lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 9 kDa

Observed band size: 12 kDa, 90 kDa




## Western blot - Anti-NEDD8 antibody [Y297] (ab81264)

NEDD8 Western blot staining of HEK293 cell lysate using rabbit Anti-NEDD8 antibody

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST
The band at 12kDa is free NEDD8, and at 90kDa is Neddylated cullins.

All lanes:
Western blot - Anti-NEDD8 antibody [Y297] (ab81264) at 1/1000 dilution

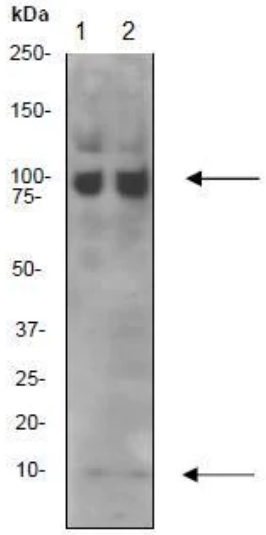
All lanes:
HEK293 cell lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 9 kDa

Observed band size: 12 kDa, 90 kDa




## Western blot - Anti-NEDD8 antibody [Y297] (ab81264)

**Other Bands:** 90kDa Neddylated cullins
All lanes:
Western blot - Anti-NEDD8 antibody [Y297] (ab81264) at 1/500 dilution

Lane 1:
HeLa cell lysate at 10 µg

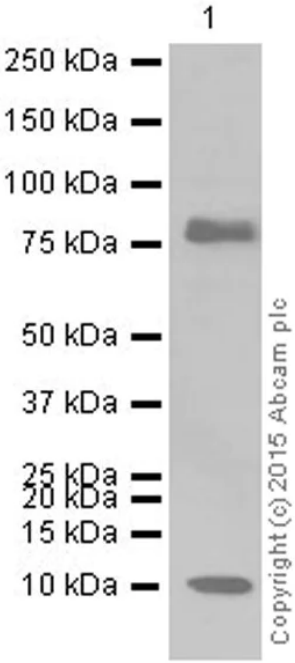
Lane 2:
K562 cell lysate at 10 µg

Secondary

All lanes:
HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 9 kDa

Observed band size: 9 kDa




## Western blot - Anti-NEDD8 antibody [Y297] (ab81264)

NEDD8 Western blot staining of PC-12 whole cell lysate using rabbit Anti-NEDD8 antibody

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST
The band at 12kDa is free NEDD8, and at 90kDa is Neddylated cullins.

All lanes:
Western blot - Anti-NEDD8 antibody [Y297] (ab81264) at 1/1000 dilution

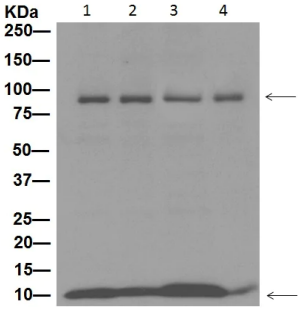
All lanes:
PC-12 whole cell lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 9 kDa

Observed band size: 12 kDa, 90 kDa




## Western blot - Anti-NEDD8 antibody [Y297] (ab81264)

All lanes:
Western blot - Anti-NEDD8 antibody [Y297] (ab81264) at 1/1000 dilution

Lane 1:
C6 Cell Lysate at 20 µg

Lane 2:
Raw264.7 Cell Lysate at 20 µg

Lane 3:
PC-12 Cell Lysate at 20 µg

Lane 4:
3T3 Cell Lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 9 kDa

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Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.