

Product datasheet

Anti-NEDD8 antibody [Y297] ab81264

Recombinant **RabMAb**

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Overview

Product name	Anti-NEDD8 antibody [Y297]
Description	Rabbit monoclonal [Y297] to NEDD8
Host species	Rabbit
Specificity	ab81264 detects NEDD8 bound to cullins proteins (neddylated cullins) as well as free NEDD8.
Tested applications	Suitable for: Flow Cyt, WB, IHC-P, ICC/IF, IP
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide within Human NEDD8 (N terminal). The exact sequence is proprietary.
Positive control	Hela and K562 cell lysates and human lung carcinoma. FC: A-673
General notes	<p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab[®] patents</p> <p>We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.</p> <p>This product is a recombinant rabbit monoclonal antibody.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	PBS 49%,Sodium azide 0.01%,Glycerol 50%,BSA 0.05%
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y297
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab81264** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt		1/300.
WB		1/1000 - 1/10000. Detects a band of approximately 9 kDa (predicted molecular weight: 9 kDa).
IHC-P		1/100 - 1/500.
ICC/IF		1/100 - 1/250.
IP		1/20 - 1/50.

Target

Function

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.

Tissue specificity

Highly expressed in heart, skeletal muscle, spleen, thymus, prostate, testis, ovary, colon and leukocytes.

Sequence similarities

Belongs to the ubiquitin family.

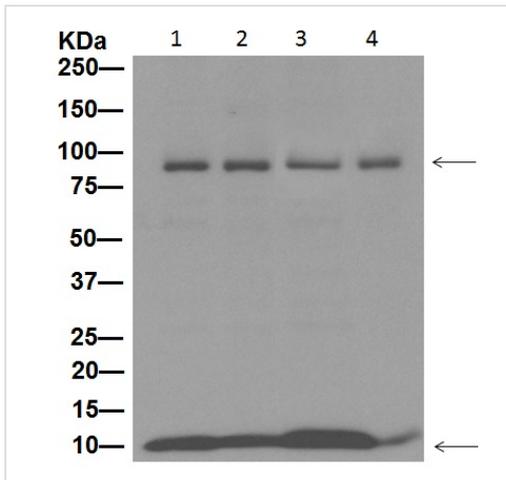
Post-translational modifications

Cleavage of precursor form by UCHL3 or SENP8 is necessary for function.

Cellular localization

Nucleus. Mainly nuclear.

Images



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

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

Lane 1 : C6 Cell Lysate

Lane 2 : Raw264.7 Cell Lysate

Lane 3 : PC-12 Cell Lysate

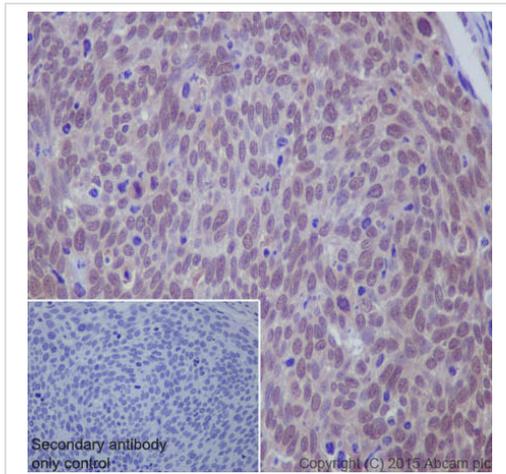
Lane 4 : 3T3 Cell Lysate

Lysates/proteins at 20 µg per lane.

Secondary

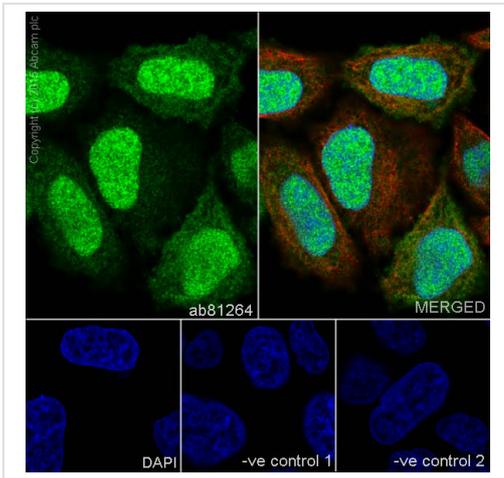
All lanes : Goat Anti-Rabbit IgG, (H+L),
Peroxidase conjugated at 1/1000 dilution

Predicted band size: 9 kDa



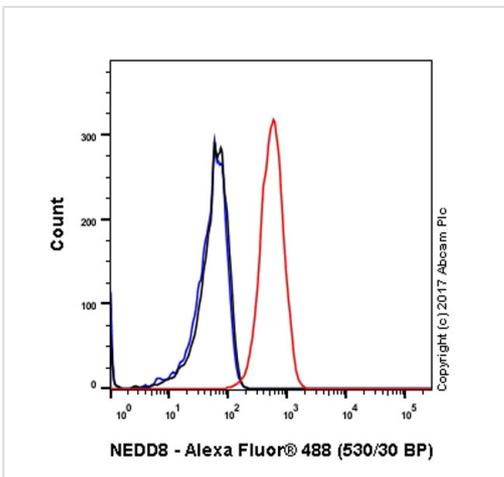
Immunohistochemistry (Formalin/PFA-fixed paraffin-
embedded sections) - Anti-NEDD8 antibody [Y297]
(ab81264)

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 performed 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.



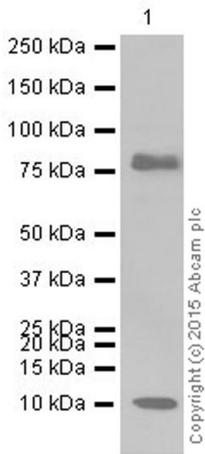
Immunocytochemistry/ Immunofluorescence - Anti-NEDD8 antibody [Y297] (ab81264)

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.



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

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 Fluor®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).



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

Anti-NEDD8 antibody [Y297] (ab81264) at
1/1000 dilution (purified) + PC-12 whole cell
lysate at 10 μ g

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at
1/20000 dilution

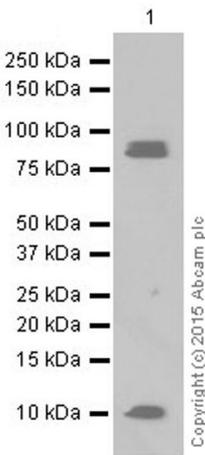
Predicted band size: 9 kDa

Observed band size: 12,90 kDa

Blocking buffer: 5% NFDm/TBST

Dilution buffer: 5% NFDm/TBST

The band at 12kDa is free NEDD8, and at
90kDa is Neddylated cullins.



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

Anti-NEDD8 antibody [Y297] (ab81264) at
1/1000 dilution (purified) + K562 whole cell
lysate at 10 μ g

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at
1/20000 dilution

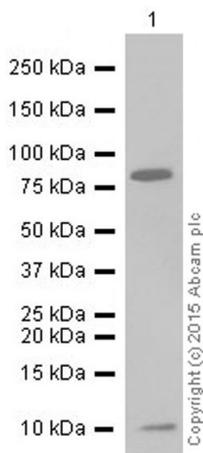
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Blocking buffer: 5% NFDm/TBST

Dilution buffer: 5% NFDm/TBST

The band at 12kDa is free NEDD8, and at
90kDa is Neddylated cullins.



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

Anti-NEDD8 antibody [Y297] (ab81264) at
1/1000 dilution (purified) + HEK293 cell lysate
at 10 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at
1/20000 dilution

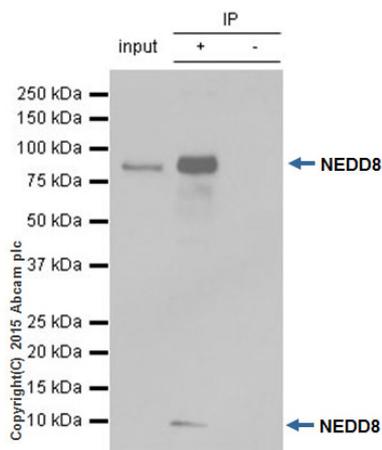
Predicted band size: 9 kDa

Observed band size: 12,90 kDa

Blocking buffer: 5% NFDm/TBST

Dilution buffer: 5% NFDm/TBST

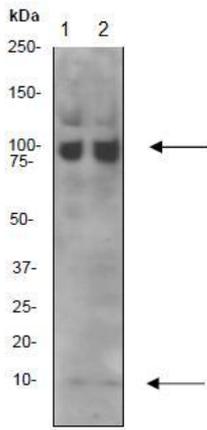
The band at 12kDa is free NEDD8, and at
90kDa is Neddylated cullins.



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, HRP
Veriblot for IP (ab131366) was used as the
secondary antibody (1/10 000). Blocking
buffer and concentration: 5% NFDm/TBST
Dilution buffer and concentration: 5%
NFDm/TBST



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

All lanes : Anti-NEDD8 antibody [Y297]
(ab81264) at 1/500 dilution (Unpurified)

Lane 1 : HeLa cell lysate

Lane 2 : K562 cell lysate

Lysates/proteins at 10 µg per lane.

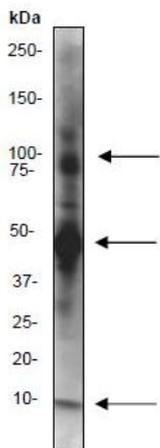
Secondary

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

Predicted band size: 9 kDa

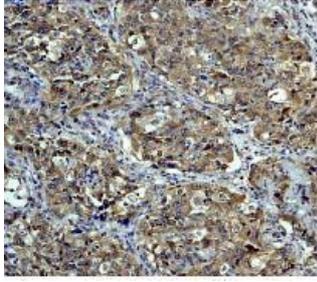
Observed band size: 9 kDa

Other Bands: 90kDa Neddylated cullins



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
fraction and 9 kDa Free NEDD8.



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

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

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