

Product datasheet

Anti-NEDD8 antibody [Y297] ab81264

Recombinant RabMAb

★★★★☆ 4 Abreviews 39 References 12 Images

Overview

Product name	Anti-NEDD8 antibody [Y297]
Description	Rabbit monoclonal [Y297] to NEDD8
Host species	Rabbit
Specificity	This antibody detects NEDD8 bound to cullins proteins (neddylated cullins) as well as free NEDD8.
Tested applications	Suitable for: Flow Cyt (Intra), 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. Flow Cyt (intra): A-673
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <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>

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	<p>pH: 7.20</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA</p>
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y297

Isotype

IgG

Applications

The Abpromise guarantee

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 (Intra)		1/300.
WB	★★★★★ (3)	1/1000 - 1/10000. Detects a band of approximately 9 kDa (predicted molecular weight: 9 kDa).
IHC-P		1/100 - 1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
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.

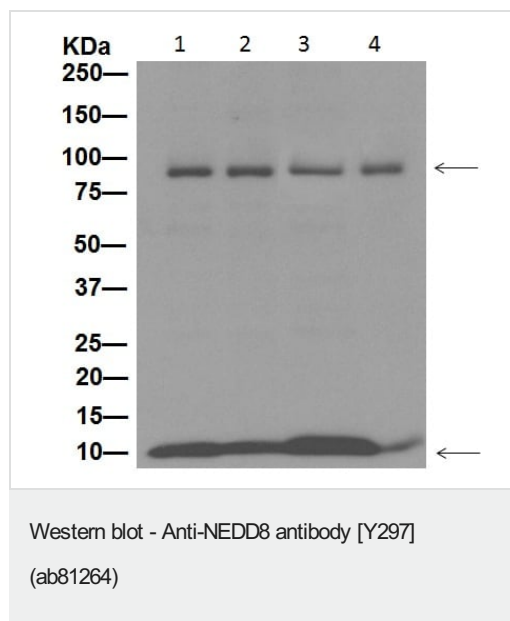
Post-translational modifications

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

Cellular localization

Nucleus. Mainly nuclear.

Images



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

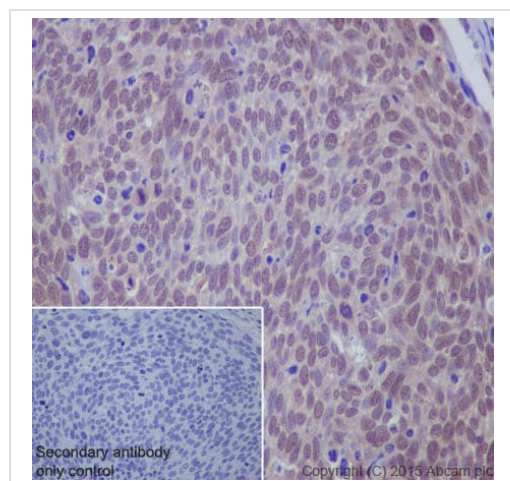
Lane 4 : 3T3 Cell Lysate

Lysates/proteins at 20 µg per lane.

Secondary

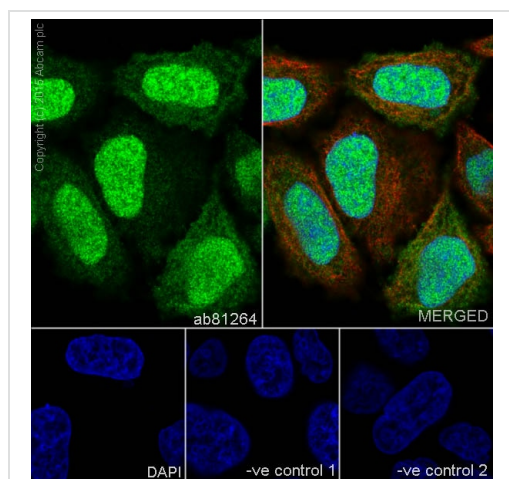
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 9 kDa



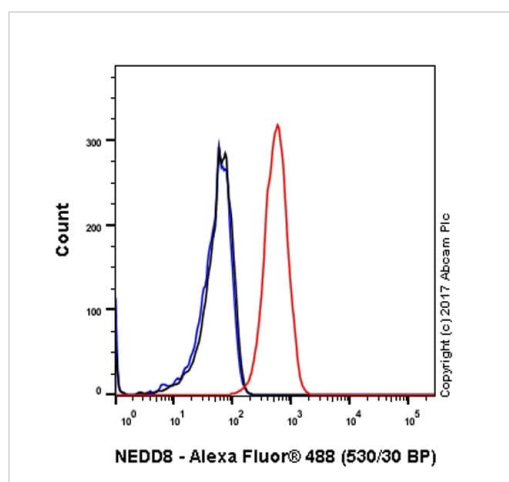
Immunohistochemical staining of paraffin embedded human cervical carcinoma with purified ab81264 at a working dilution of 1/400. The secondary antibody used is **ab97051**, a goat anti-rabbit IgG (H&L) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed 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.

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



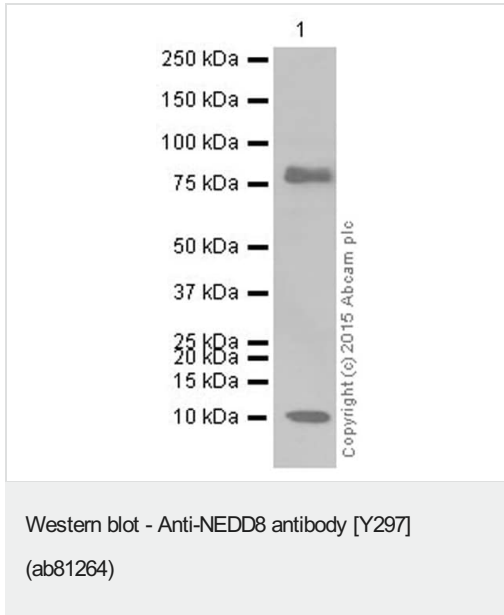
Immunocytochemistry/ Immunofluorescence - Anti-NEDD8 antibody [Y297] (ab81264)

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 (Intracellular) - Anti-NEDD8 antibody [Y297] (ab81264)

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 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).



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

Secondary

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

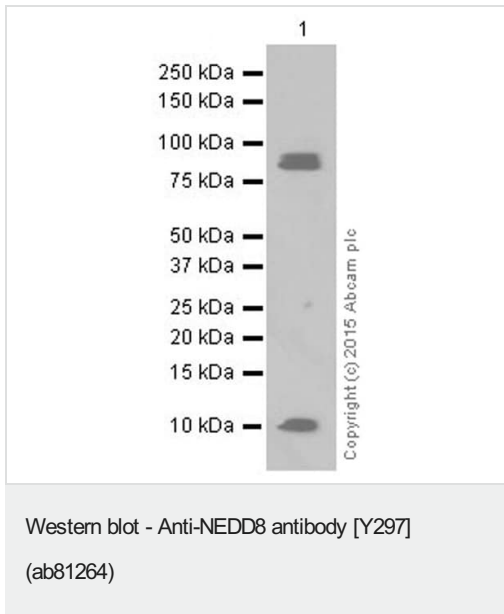
Predicted band size: 9 kDa

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.



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

Secondary

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

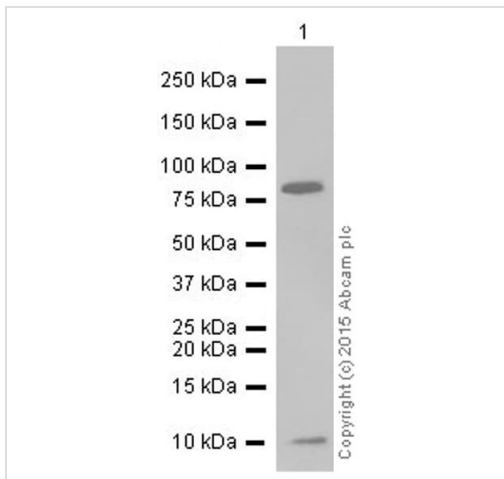
Predicted band size: 9 kDa

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.



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

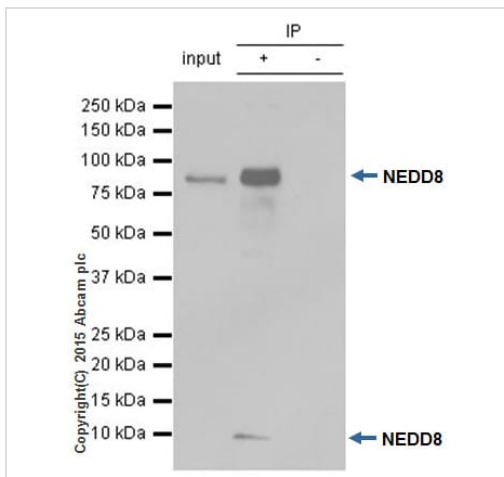
Predicted band size: 9 kDa

Observed band size: 12,90 kDa

Blocking buffer: 5% NFDM/TBST

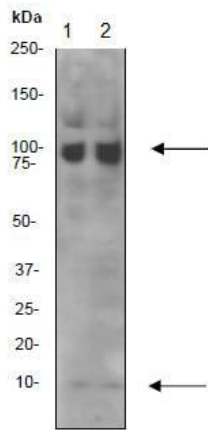
Dilution buffer: 5% NFDM/TBST

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,
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



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.

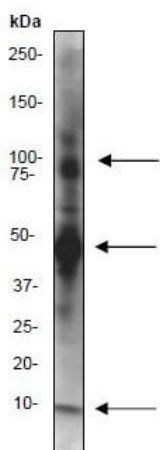
Secondary

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

Predicted band size: 9 kDa

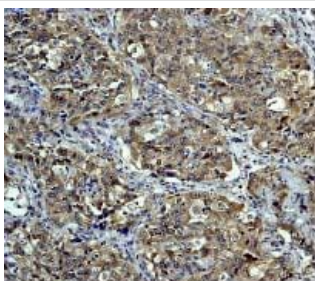
Observed band size: 9 kDa

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.



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

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

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-NEDD8 antibody [Y297] (ab81264)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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