# abcam

### Product datasheet

## Anti-NEDD8 antibody [Y297] ab81264



重组 RabMAb

★★★★★ 4 Abreviews 39 References 12 图像

概述

产品名称 Anti-NEDD8抗体[Y297]

描述 兔单克隆抗体[Y297] to NEDD8

宿主 Rabbit

特异性 This antibody detects NEDD8 bound to cullins proteins (neddylated cullins) as well as free

经测试应用 适用于: Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP

种属反应性 与反应: Mouse, Rat, Human

免疫原 Synthetic peptide within Human NEDD8 (N terminal). The exact sequence is proprietary.

阳性对照 Hela and K562 cell lysates and human lung carcinoma. Flow Cyt (intra): A-673

This product is a recombinant monoclonal antibody, which offers several advantages including: 常规说明

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

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

性能

形式 Liquid

存放说明 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.

存储溶液 pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA

纯度 Protein A purified

单克降 克降 克隆编号 Y297

**同种型** lgG

### 应用

### The Abpromise guarantee Abpromise™承诺保证使用ab81264于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
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.

功能	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.
组织 <b>特异性</b>	Highly expressed in heart, skeletal muscle, spleen, thymus, prostate, testis, ovary, colon and leukocytes.
序列相似性	Belongs to the ubiquitin family.

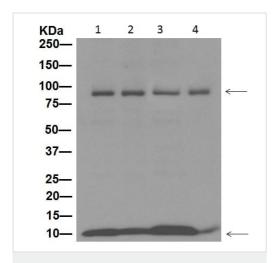
Cleavage of precursor form by UCHL3 or SENP8 is necessary for function.

细胞定位 Nucleus. Mainly nuclear.

图片

翻译后修饰

靶标



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

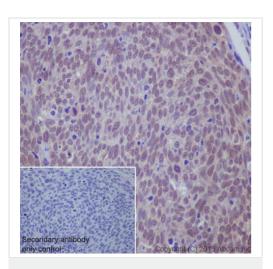
Lane 4: 3T3 Cell Lysate

Lysates/proteins at 20 µg per lane.

### **Secondary**

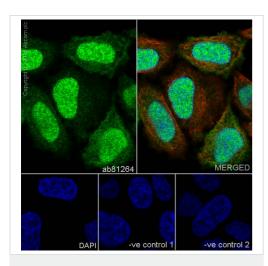
**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 9 kDa

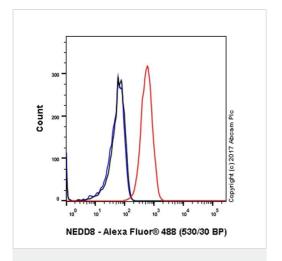


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NEDD8 antibody [Y297] (ab81264)

Immunohistochemical staining of paraffin embedded human cervical carcinoma with purified ab81264 at a working dilution of 1/400. The secondary antibody used is <a href="mailto:ab97051">ab97051</a>, 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.



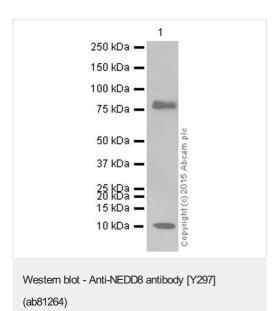
Immunocytochemistry/ Immunofluorescence - Anti-NEDD8 antibody [Y297] (ab81264)



Flow Cytometry (Intracellular) - 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 antitubulin 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 antitubulin) 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.

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<sup>®</sup>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 $\mu g$

### **Secondary**

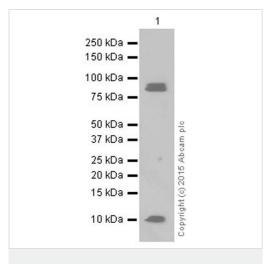
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

**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 µg

### **Secondary**

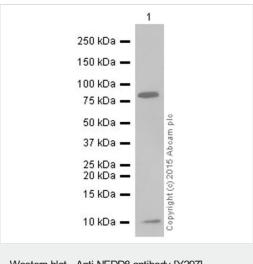
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

**Predicted band size:** 9 kDa **Observed band size:** 12,90 kDa

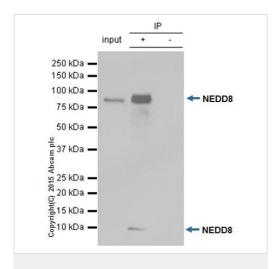
Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST

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

cullins.



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



Immunoprecipitation - 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.

ab81264 (purified) at 1/20 immunoprecipitating NEDD8 in 10  $\mu$ g K562 cell lysate (Lanes 1 and 2, observed at 10 and 90 kDa). Lane 3 - Rabbit monoclonal lgG (<u>ab172730</u>). For western blotting, VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), 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

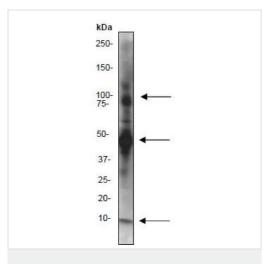
Lysates/proteins at 10 µg per lane.

**Secondary** 

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

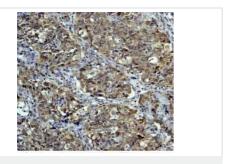
**Predicted band size:** 9 kDa **Observed band size:** 9 kDa

Other Bands: 90kDa Neddylated cullins



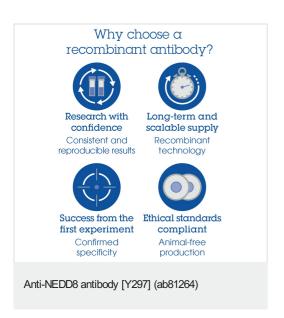
Immunoprecipitation - Anti-NEDD8 antibody [Y297] (ab81264)

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 lgG fraaction and 9 kDa Free NEDD8.



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

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



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