

### Anti-NEDD8 antibody [Y297] ab81264

リコンビナント RabMAb

★★★★☆ **4 Abreviews** **39 References** **画像数 12**

#### 製品の概要

製品名	Anti-NEDD8 antibody [Y297]
製品の詳細	Rabbit monoclonal [Y297] to NEDD8
由来種	Rabbit
特異性	This antibody detects NEDD8 bound to cullins proteins (neddylated cullins) as well as free NEDD8.
アプリケーション	<b>適用あり:</b> Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP
種交差性	<b>交差種:</b> Mouse, Rat, Human
免疫原	Synthetic peptide within Human NEDD8 (N terminal). The exact sequence is proprietary.
ポジティブ・コントロール	Hela and K562 cell lysates and human lung carcinoma. Flow Cyt (intra): A-673
特記事項	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"><li>- High batch-to-batch consistency and reproducibility</li><li>- Improved sensitivity and specificity</li><li>- Long-term security of supply</li><li>- Animal-free production</li></ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

#### 製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	Y297

## アプリケーション

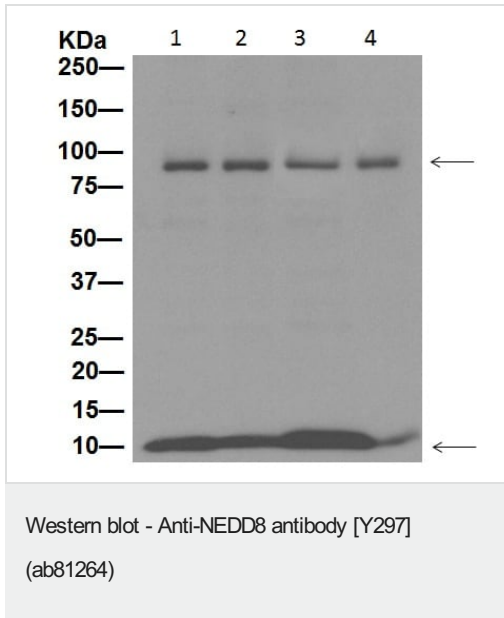
The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab81264の使用に適用されます**  
 アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
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.

## ターゲット情報

機能	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.
組織特異性	Highly expressed in heart, skeletal muscle, spleen, thymus, prostate, testis, ovary, colon and leukocytes.
配列類似性	Belongs to the ubiquitin family.
翻訳後修飾	Cleavage of precursor form by UCHL3 or SENP8 is necessary for function.
細胞内局在	Nucleus. Mainly nuclear.

## 画像



**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

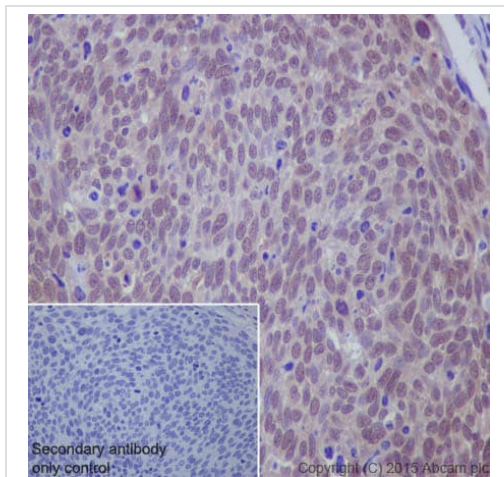
**Lane 4** : 3T3 Cell Lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

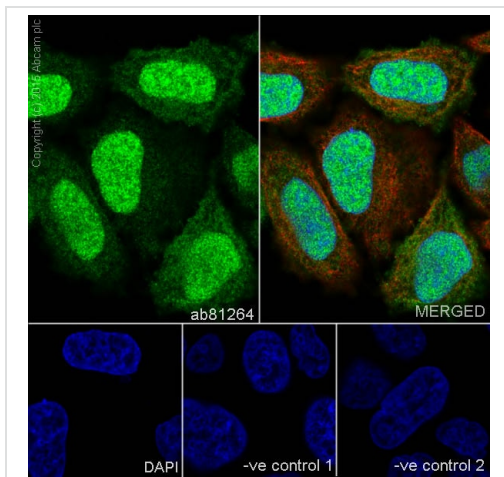
**All lanes** : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

**Predicted band size:** 9 kDa



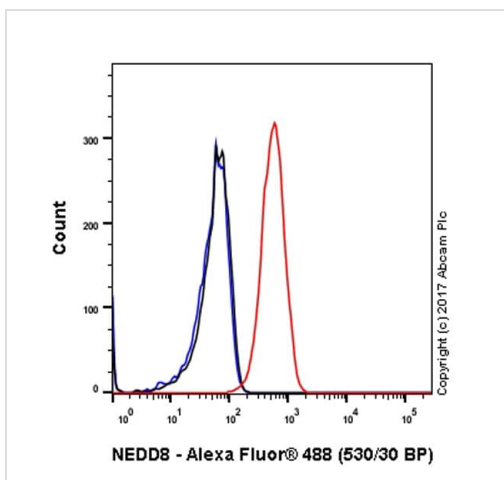
Immunohistochemical staining of paraffin embedded human cervical carcinoma with purified ab81264 at a working dilution of 1/400. The secondary antibody used is [ab97051](#), a goat anti-rabbit IgG (H&L) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed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



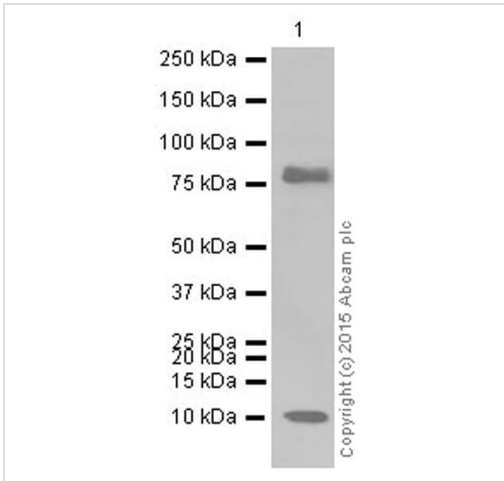
Immunocytochemistry/ Immunofluorescence - Anti-NEDD8 antibody [Y297] (ab81264)

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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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<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).



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

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

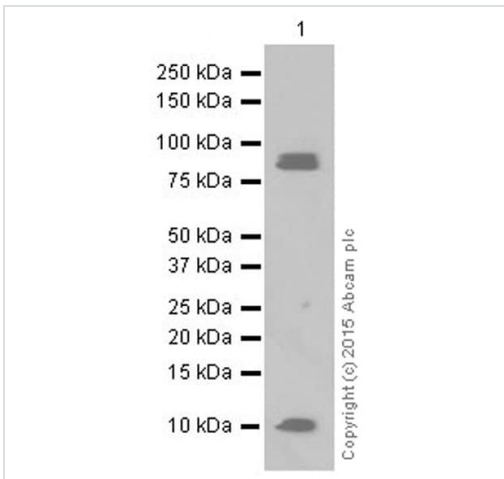
**Predicted band size:** 9 kDa

**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.



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

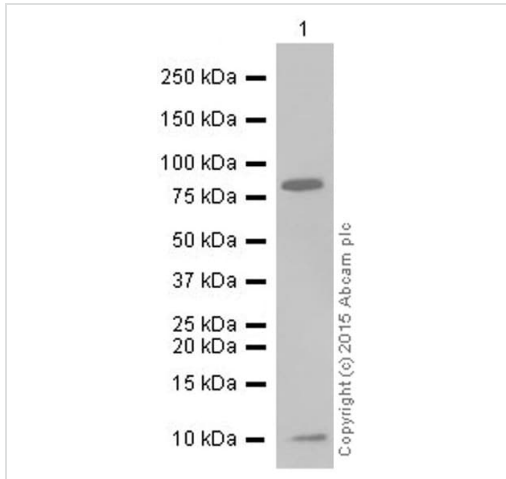
**Predicted band size:** 9 kDa

**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)

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

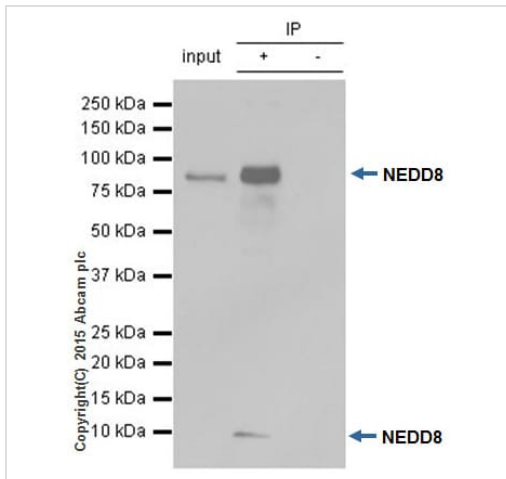
**Predicted band size:** 9 kDa

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

Blocking buffer: 5% NFDm/TBST

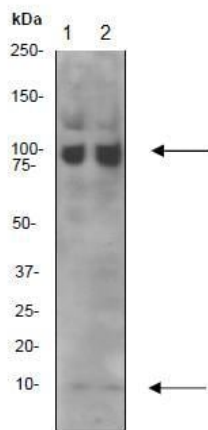
Dilution buffer: 5% NFDm/TBST

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.

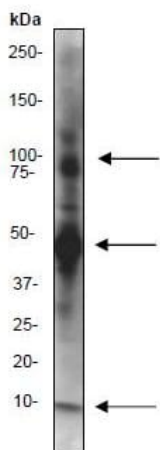
**Secondary**

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

**Predicted band size:** 9 kDa

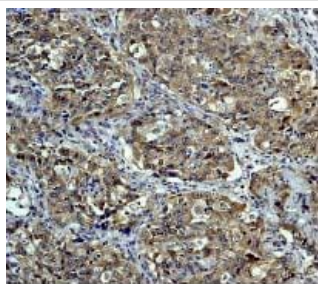
**Observed band size:** 9 kDa

**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)

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