abcam

Product datasheet

Anti-OTUB1 antibody [EPR13028(B)] ab175200





重组 RabMAb

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概述

产品名称 Anti-OTUB1抗体[EPR13028(B)]

描述 兔单克隆抗体[EPR13028(B)] to OTUB1

宿主 Rabbit

经测试应用 适用于: WB, IP

不适用于: Flow Cyt,ICC/IF or IHC-P

种属反应性 与反应: Mouse, Rat, Human

免疫原 Synthetic peptide within Human OTUB1 aa 200 to the C-terminus (Cysteine residue). The exact

> sequence is proprietary. Database link: Q96FW1

阳性对照 WB: Wild-type HAP1, HeLa, MCF7, HepG2, HEK-293T, and HEK-293 cell lysates. Rat and

mouse heart tissue lysates. IP: HeLa cell lysate.

常规说明 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 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 long

term. Avoid freeze / thaw cycle.

存储溶液 Preservative: 0.01% Sodium azide

Constituents: PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)

纯度 Protein A purified

单克隆 克隆

克隆编号 EPR13028(B)

同种型 IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab175200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
WB	*** <u>*</u> (2)	1/1000 - 1/10000. Predicted molecular weight: 31 kDa.
IP	*** <u>*</u>	1/10 - 1/100.

应用说明

Is unsuitable for Flow Cyt,ICC/IF or IHC-P.

靶标

功能

Hydrolase that can remove conjugated ubiquitin from proteins and plays an important regulatory role at the level of protein turnover by preventing degradation. Regulator of T-cell anergy, a phenomenon that occurs when T-cells are rendered unresponsive to antigen rechallenge and no longer respond to their cognate antigen. Acts via its interaction with RNF128/GRAIL, a crucial inductor of CD4 T-cell anergy. Isoform 1 destabilizes RNF128, leading to prevent anergy. In contrast, isoform 2 stabilizes RNF128 and promotes anergy. Surprisingly, it regulates RNF128-mediated ubiquitination, but does not deubiquitinate polyubiquitinated RNF128. Deubiquitinates estrogen receptor alpha (ESR1). Mediates deubiquitination of 'Lys-48'-linked polyubiquitin chains, but not 'Lys-63'-linked polyubiquitin chains. Not able to cleave di-ubiquitin. Also capable of removing NEDD8 from NEDD8 conjugates, but with a nuch lower preference compared to 'Lys-48'-linked ubiquitin.

组织特异性

lsoform 1 is ubiquitous. Isoform 2 is expressed only in lymphoid tissues such as tonsils, lymph nodes and spleen, as well as peripheral blood mononuclear cells.

序列相似性

Belongs to the peptidase C65 family.

Contains 1 OTU domain.

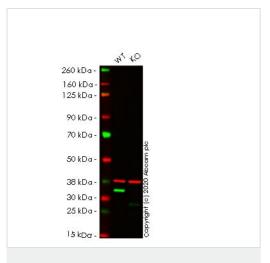
结构域

In addition to ubiquitin-binding at the Cys-91 active site, a proximal ubiquitin-binding site is also present at Cys-23 Occupancy of the active site is needed to enable tight binding to the second site. Distinct binding sites for the ubiquitins may allow to discriminate among different isopeptide linkages (i.e. 'Lys-48'-, 'Lys-63'-linked polyubiquitin) in polyubiquitin substrates and achieve linkage-specific deubiquitination.

Cytoplasm.

细胞定位

图片



Western blot - Anti-OTUB1 antibody [EPR13028(B)] (ab175200)

All lanes : Anti-OTUB1 antibody [EPR13028(B)] (ab175200) at 1/1000 dilution

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: OTUB1 knockout HEK-293T cell lysate

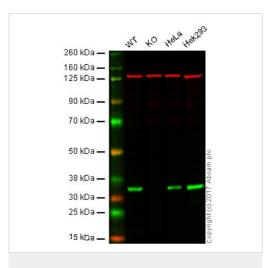
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 31 kDa Observed band size: 31 kDa

Lanes 1-2: Merged signal (red and green). Green - ab175200 observed at 31 kDa. Red - loading control <u>ab8245</u> observed at 37 kDa.

ab175200 Anti-OTUB1 antibody [EPR13028(B)] was shown to specifically react with OTUB1 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab266551 (knockout cell lysate ab257569) was used. Wild-type and OTUB1 knockout samples were subjected to SDS-PAGE. ab175200 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°CC at 1 in 1000 Dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-OTUB1 antibody [EPR13028(B)] (ab175200)

All lanes : Anti-OTUB1 antibody [EPR13028(B)] (ab175200) at 1/1000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate

Lane 2: OTUB1 knockout HAP1 whole cell lysate

Lane 3: HeLa whole cell lysate

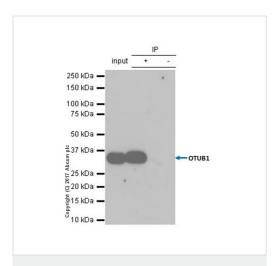
Lane 4: HEK-293 whole cell lysate

Lysates/proteins at 20 µg per lane.

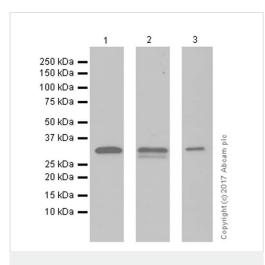
Predicted band size: 31 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab175200 observed at 35 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

ab175200 was shown to specifically react with OTUB1 in wild type cells as signal was lost in OTUB1 knockout cells. Wild-type and OTUB1 knockout samples were subjected to SDS-PAGE. ab175200 and ab18058 (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunoprecipitation - Anti-OTUB1 antibody [EPR13028(B)] (ab175200)



Western blot - Anti-OTUB1 antibody [EPR13028(B)] (ab175200)

ab175200 (purified) at 1:50 dilution (2ug) immunoprecipitating OTUB1 in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate.

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10ug

Lane 2 (+): ab175200 & HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab175200 in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.

All lanes : Anti-OTUB1 antibody [EPR13028(B)] (ab175200) at 1/1000 dilution (purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lane 2: Mouse heart whole tissue lysates

Lane 3: Rat heart whole tissue lysates

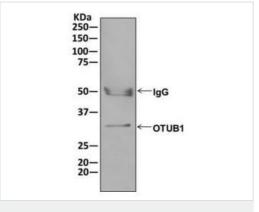
Lysates/proteins at 15 µg per lane.

Secondary

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

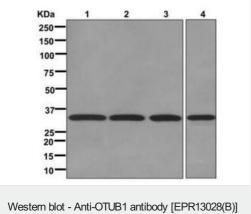
Predicted band size: 31 kDa **Observed band size:** 31 kDa

Blocking and diluting buffer: 5% NFDM/TBST



Western blot analysis on immunoprecipitation pellet from MCF7 cell lysate labeling OTUB1 with unpurified ab175200 at 1/10 dilution.

Immunoprecipitation - Anti-OTUB1 antibody [EPR13028(B)] (ab175200)



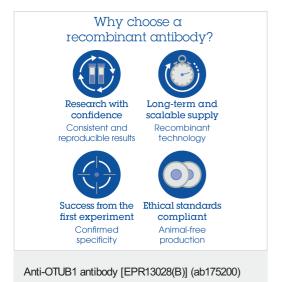
Western blot - Anti-OTUB1 antibody [EPR13028(B)] (ab175200)

All lanes : Anti-OTUB1 antibody [EPR13028(B)] (ab175200) at 1/1000 dilution (unpurified)

Lane 1 : HeLa cell lysate
Lane 2 : HepG2 cell lysate
Lane 3 : 293T cell lysate
Lane 4 : MCF7 cell lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 31 kDa



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