abcam

Product datasheet

Anti-RIP antibody [EPR24883-85] ab300617

敲除验证 重组 RabMAb

1 References 9 图像

概述

产品名称	Anti-RIP抗体[EPR24883-85]
描述	兔单克隆抗体[EPR24883-85] to RIP
宿主	Rabbit
特异性	Not suitable for mouse and rat IHC-P.
经测试应用	**适用于:** WB, IHC-P, IP **不适用于:** Flow Cyt (Intra) or ICC/IF
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: Wild-type and RIP knockout HAP1 whole cell lysate; HeLa, 293T, NIH/3T3, PC-12 whole cell lysates; rat and mouse testis tissue lysates. IHC-P: Human cervical carcinoma FFPE tissue sections; Wild-type and RIP knockout HAP1 cell pellets. IP: HeLa whole cell lysate, NIH/3T3 whole 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	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified

克隆	单克隆
克隆编号	EPR24883-85
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab300617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		1/1000. Detects a band of approximately 75 kDa (predicted molecular weight: 75 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/30.

应用说明 Is unsuitable for Flow Cyt (Intra) or ICC/IF.

靶标

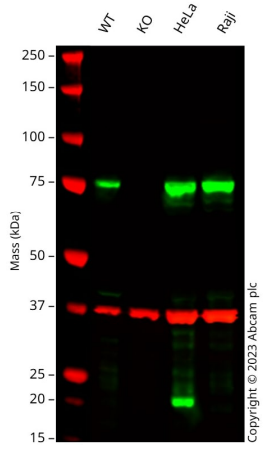
功能 Essential adapter molecule for the activation of NF-kappa-B. Following different upstream signals (binding of inflammatory cytokines, stimulation of pathogen recognition receptors, or DNA damage), particular RIPK1-containing complexes are formed, initiating a limited number of cellular responses. Upon TNFA stimulation RIPK1 is recruited to a TRADD-TRAF complex initiated by TNFR1 trimerization. There, it is ubiquitinated via 'Lys-63'-link chains, inducing its association with the IKK complex, and its activation through NEMO binding of polyubiquitin chains.

序列相似性 Belongs to the protein kinase superfamily. TKL Ser/Thr protein kinase family.
Contains 1 death domain.
Contains 1 protein kinase domain.

翻译后修饰 Proteolytically cleaved by caspase-8 during TNF-induced apoptosis. Cleavage abolishes NF-kappa-B activation and enhances pro-apototic signaling through the TRADD-FADD interaction.
Autophosphorylated on serine and threonine residues.
Ubiquitinated by 'Lys-11'-, 'Lys-48'-, 'Lys-63'- and linear-linked type ubiquitin. Polyubiquitination with 'Lys-63'-linked chains by TRAF2 induces association with the IKK complex. Deubiquitination of 'Lys-63'-linked chains and polyubiquitination with 'Lys-48'-linked chains by TNFAIP3 leads to RIPK1 proteasomal degradation and consequently to the termination of the TNF- or Linear polyubiquitinated; the head-to-tail polyubiquitination is mediated by the LUBAC complex. LPS-mediated activation of NF-kappa-B. Also ubiquitinated with 'Lys-11'-linked chains.

细胞定位 Cytoplasm.

图片



Western blot - Anti-RIP antibody [EPR24883-85] (ab300617)

All lanes : Anti-RIP antibody [EPR24883-85] (ab300617) at 1/1000 dilution

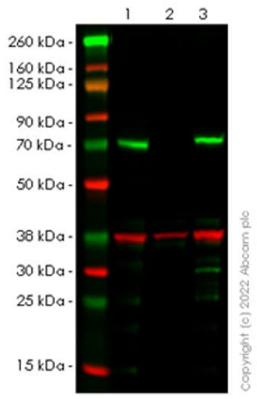
Lane 1 : Wild-type THP-1 cell lysate
Lane 2 : RIPK1 knockout THP-1 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : Raji cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 75 kDa
Observed band size: 75 kDa

Anti-RIPK1 antibody [EPR24883-85] (ab300617) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab300617 was shown to bind specifically to RIPK1. A band was observed at 75 kDa in wild-type THP-1 cell lysates with no signal observed at this size in RIPK1 knockout cell line **ab276121** (knockout cell lysate **ab284221**). To generate this image, wild-type and RIPK1 knockout THP-1 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween$^®$ 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution



Western blot - Anti-RIP antibody [EPR24883-85] (AB300617)

All lanes : Anti-RIP antibody [EPR24883-85] (ab300617) at 1/1000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : RIP knockout HAP1 whole cell lysate
Lane 3 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (**ab216773**) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (**ab216776**) at 1/10000 dilution

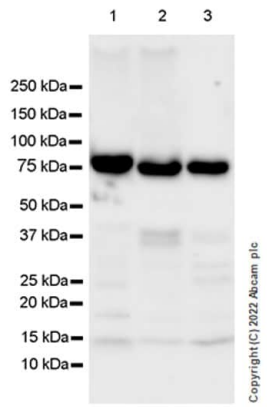
Predicted band size: 75 kDa
Observed band size: 75 kDa

False colour image of Western blot: Anti-RIP antibody [EPR24883-85] (ab300617) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red.

Blocking / Diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS

WB performed under reducing conditions.

ab300617 was shown to bind specifically to RIP. A band was observed at 75 kDa in wild-type HAP1 cell lysates with no signal observed at this size in RIP knockout cell line. To generate this image, wild-type and RIP knockout HAP1 cell lysates were analyzed. First, samples were run on an SDS-PAGE gel then transferred onto an immobilon-FL PVDF membrane. Membranes were blocked in in Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged.



Western blot - Anti-RIP antibody [EPR24883-85] (AB300617)

All lanes : Anti-RIP antibody [EPR24883-85] (ab300617) at 1/1000 dilution

Lane 1 : 293T (human embryonic kidney epithelial cell) whole cell lysate
Lane 2 : NIH/3T3 (mouse embryonic fibroblast) whole cell lysate
Lane 3 : PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

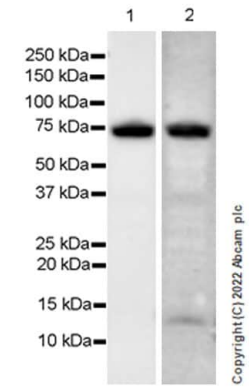
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 75 kDa
Observed band size: 75 kDa

Exposure time: 15 seconds

Blocking / Diluting buffer and concentration: 5% NFDM/TBST

Lysates were freshly made and used for Western blotting immediately to minimize protein degradation.



Western blot - Anti-RIP antibody [EPR24883-85] (AB300617)

All lanes : Anti-RIP antibody [EPR24883-85] (ab300617) at 1/1000 dilution

Lane 1 : Mouse testis tissue lysate

Lane 2 : Rat testis tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

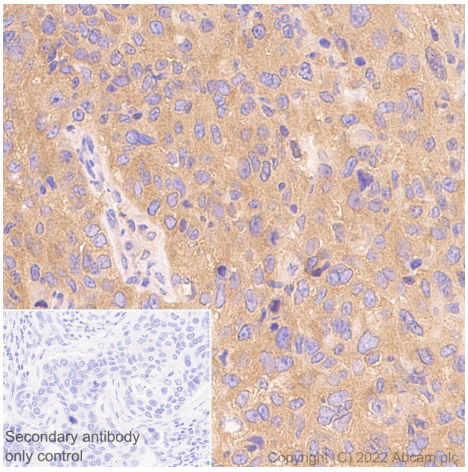
Predicted band size: 75 kDa

Observed band size: 75 kDa

Blocking / Diluting buffer and concentration: 5% NFDM/TBST

Exposure time:

Lane 1: 37 seconds; lane 2: 180 seconds.

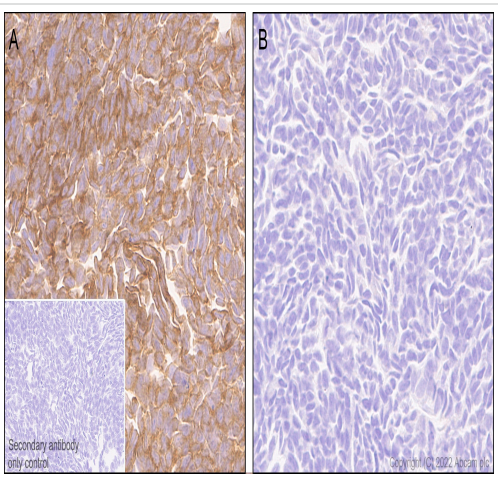


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RIP antibody [EPR24883-85] (AB300617)

Immunohistochemical analysis of paraffin-embedded human cervical carcinoma tissue labelling RIP with ab300617 at 1/100 dilution (5.11 µg/mL) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Cytoplasmic staining on human cervical carcinoma was observed. The section was incubated with ab300617 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0 epitope retrieval solution 2) for 20 mins.

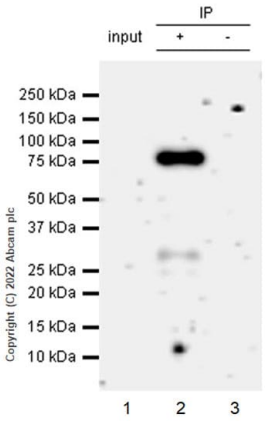


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RIP antibody [EPR24883-85] (AB300617)

Immunohistochemical analysis of paraffin-embedded Wild-type and RIPK1 knockout HAP1 cells labelling RIP with ab300617 at 1/100 dilution (5.11 µg/mL) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on Wild-type HAP1 cell pellet (A), and no staining on RIPK1 knockout HAP1 cell pellet (B) were observed. The section was incubated with ab300617 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0 epitope retrieval solution 2) for 20 mins.



Immunoprecipitation - Anti-RIP antibody [EPR24883-85] (AB300617)

RIP was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate with ab300617 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab300617 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(**ab131366**) was used at 1/5000 dilution.

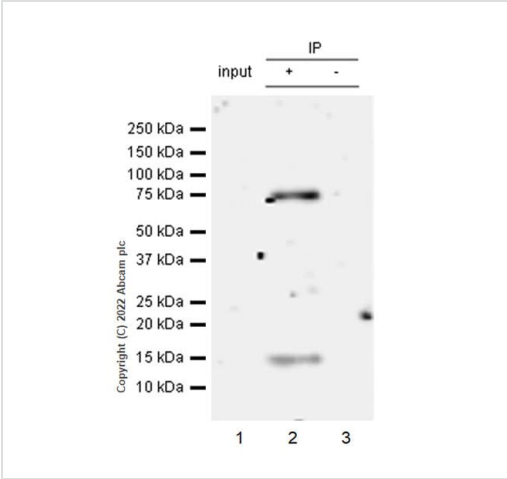
Lane 1: HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg (Input)

Lane 2: ab300617 IP in HeLa whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab300617 in HeLa whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 41 seconds



Immunoprecipitation - Anti-RIP antibody [EPR24883-85] (AB300617)

RIP was immunoprecipitated from 0.35 mg NIH/3T3 (mouse embryonic fibroblast) whole cell lysate with ab300617 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab300617 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(**ab131366**) was used at 1/5000 dilution.

Lane 1: NIH/3T3 (mouse embryonic fibroblast) whole cell lysate 10 µg (Input)

Lane 2: ab300617 IP in NIH/3T3 whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab300617 in NIH/3T3 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes

Anti-RIP antibody [EPR24883-85] (AB300617)

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