# abcam

## Product datasheet

## Anti-RIP antibody [EPR4689-100] - BSA and Azide free ab227843





重组 RabMAb

## 4 图像

### 概述

产品名称 Anti-RIP抗体[EPR4689-100] - BSA and Azide free

描述 兔单克隆抗体[EPR4689-100] to RIP - BSA and Azide free

宿主 Rabbit

经测试应用 适用于: Flow Cyt (Intra), WB

不适用于: ICC/IF,IHC-P or IP

种属反应性 与反应: Human

免疫原 Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

阳性对照 HeLa cells and cell lysates; Raji cell lysates.

常规说明 ab227843 is the carrier-free version of ab178420.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

## 性能

形式 Liquid

存放说明 Shipped at 4°C. Store at +4°C. Do Not Freeze.

**存储溶液** pH: 7.20

Constituent: PBS

**无载体** 是

纯**度** Protein A purified

**克隆** 单克隆

**克隆编号** EPR4689-100

**同种型** IgG

#### 应用

## The Abpromise guarantee Abpromise™承诺保证使用ab227843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)		Use at an assay dependent concentration. <b>ab199376</b> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Predicted molecular weight: 75 kDa.

应用说明 Is unsuitable for ICC/IF,IHC-P or IP.

#### 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-

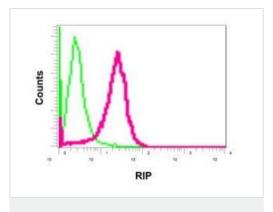
kappa-B activation and enhances pro-apototic signaling through the TRADD-FADD interaction.

Autophosphorylated on serine and threonine residues.

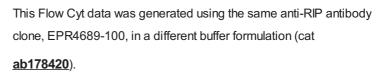
Ubiquitinated by 'Lys-11'-, 'Lys-48'-, 'Lys-63'- and linear-linked type ubiquitin. Polyubiquitination with 'Lys-63'-linked chains by TRAF2 induces association with the IKK complex. Deubiquitination of 'Lys-63'-linked chains and polyubiquitination with 'Lys-48'-linked chains by TNFAIP3 leads to RIPK1 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.

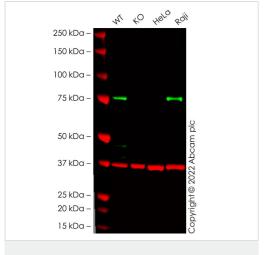
细**胞定位** Cytoplasm.



Flow Cytometry (Intracellular) - Anti-RIP antibody [EPR4689-100] - BSA and Azide free (ab227843)



Intracellular flow cytometric analysis of permeabilized HeLa cells labeling RIP with <u>ab178420</u> at 1/10 dilution (red) compared with a rabbit lgG negative control (green).



Western blot - Anti-RIP antibody [EPR4689-100] - BSA and Azide free (ab227843)

**All lanes :** Anti-RIP antibody [EPR4689-100] (<u>ab178420</u>) 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:** 76 kDa

False colour image of Western blot: Anti-RIP antibody [EPR4689-100] 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, ab178420 was shown to bind specifically to RIP. A band was observed at 76 kDa in wild-type THP-1 cell lysates with no signal observed at this size in RIPK1 knockout cell line ab276121 (knockout cell lysate ab284210). 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<sup>®</sup> 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  $\lg G \ H\&L \ (lRDye^{\&}\ 800CW)$  preabsorbed  $(\underline{ab216773})$  and Goat anti-Mouse  $\lg G \ H\&L \ (lRDye^{\&}\ 680RD)$  preabsorbed  $(\underline{ab216776})$  at 1/20000 dilution.

250 kDa —

150 kDa —

100 kDa —

75 kDa —

37 kDa —

25 kDa —

20 kDa —

15 kDa —

10 kDa —

10 kDa —

Western blot - Anti-RIP antibody [EPR4689-100] -

BSA and Azide free (ab227843)

This WB data was generated using the same anti-RIP antibody clone, EPR4689-100, in a different buffer formulation (cat# ab178420).

Lane 1: Wild-type HAP1 cell lysate (20 µg)

Lane 2: RIP knockout HAP1 cell lysate (20 µg)

Lane 3: HeLa cell lysate (20 µg)

Lane 4: Raji cell lysate (20 µg)

**Lanes 1 to 4:** Merged signal (red and green). Green - <u>ab178420</u> observed at 78 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

ab178420 was shown to specifically react with RIP when RIP knockout samples were used. Wild-type and RIP knockout samples were subjected to SDS-PAGE. ab178420 and ab8245 (loading control to GAPDH) were both diluted 1/1000 and 1/10000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) ab216776 secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.



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