abcam

Product datasheet

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

KO VALIDATED Recombinant RabMAb

4 Images

Overview

Product name	Anti-RIP antibody [EPR4689-100] - BSA and Azide free
Description	Rabbit monoclonal [EPR4689-100] to RIP - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB **Unsuitable for:** ICC/IF,IHC-P or IP
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	HeLa cells and cell lysates; Raji cell lysates.
General notes	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 cell-based 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® 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.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR4689-100
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab227843 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

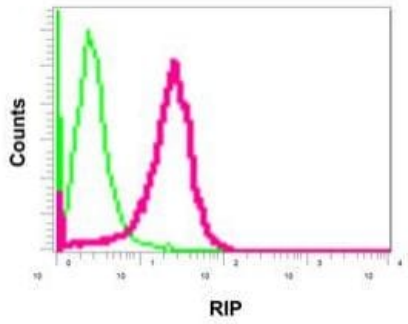
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Predicted molecular weight: 75 kDa.

Application notes Is unsuitable for ICC/IF,IHC-P or IP.

Target

Function	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.
Sequence similarities	Belongs to the protein kinase superfamily. TKL Ser/Thr protein kinase family. Contains 1 death domain. Contains 1 protein kinase domain.
Post-translational modifications	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.
Cellular localization	Cytoplasm.

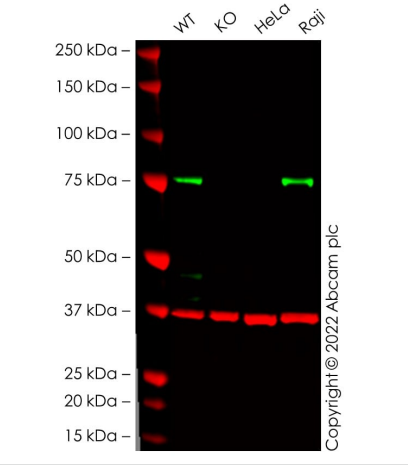
Images



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

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

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



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

All lanes : Anti-RIP antibody [EPR4689-100] (**ab178420**) 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

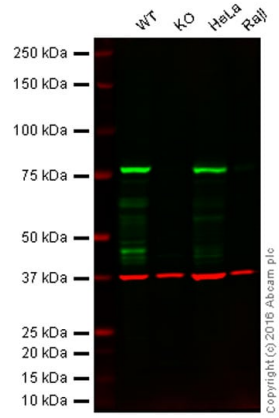
Lysates/proteins at 20 µg per lane.

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® 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 (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



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 - **ab178420** observed at 78 kDa. Red - loading control, **ab8245**, 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.

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

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