abcam

Product datasheet

Anti-RIP antibody [EPR4689] ab125072



Recombinant RabMAb

4 References 6 Images

Overview

Product name Anti-RIP antibody [EPR4689]

Description Rabbit monoclonal [EPR4689] to RIP

Host species Rabbit

Suitable for: WB **Tested applications**

Unsuitable for: IHC-P

Reacts with: Human Species reactivity

Recombinant fragment corresponding to Human RIP aa 300-450 (internal sequence). **Immunogen**

Positive control WB: Raji, Jurkat, HeLa and 293T cell lysates

General notes 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.

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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number EPR4689

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise quarantee covers the use of ab125072 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
WB		1/1000 - 1/10000. Predicted molecular weight: 75 kDa.

Application notes

Is unsuitable for IHC-P.

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.

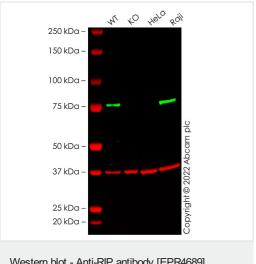
Autophosphorylated on serine and threonine residues.

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



Western blot - Anti-RIP antibody [EPR4689] (ab125072)

All lanes : Anti-RIP antibody [EPR4689] (ab125072) 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

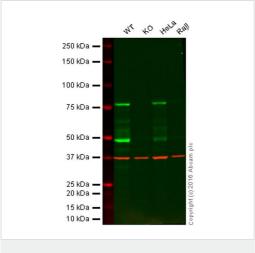
Lane 4 : Raji cell lysate

Lysates/proteins at 20 µg per lane.

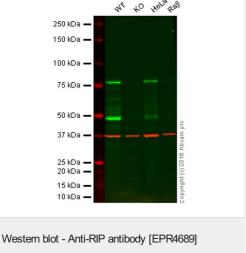
Performed under reducing conditions.

Predicted band size: 75 kDa **Observed band size:** 76 kDa

False colour image of Western blot: Anti-RIP antibody [EPR4689] 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, ab125072 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.



(ab125072)



Anti-RIP antibody [EPR4689] (ab125072) at 1/5000 dilution (Purified) + Raji (Human Burkitt's lymphoma B lymphocyte) whole cell lysate at 15 µg

250 kDa -150 kDa -100 kDa -75 kDa -RIF 50 kDa -Copyright (c) 2018 Aboam plo 37 kDa 🕳 25 kDa — 20 kDa — 15 kDa -10 kDa -

Western blot - Anti-RIP antibody [EPR4689] (ab125072)

Secondary

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

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

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

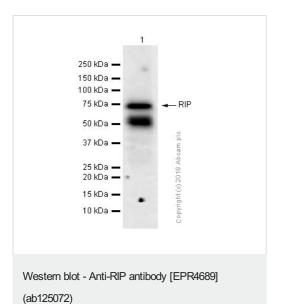
Lane 2: RIP knockout HAP1 cell lysate (20 µg)

Lane 3: HeLa cell lysate (20 µg)

Lane 4: Raji cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab125072 (unpurified) observed at 78 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab125072 was shown to specifically react with RIP in wild-type HAP1 cells. No band was observed when RIP knockout samples were examined. Wild-type and RIP knockout samples were subjected to SDS-PAGE. ab125072 at a dilution of 1/1000 and ab8245 (loading control to GAPDH) at a dilution of 1/10,000 were incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.

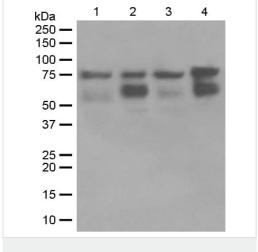


Anti-RIP antibody [EPR4689] (ab125072) at 1/1000 dilution (Purified) + HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates at 15 μg

Secondary

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

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



Western blot - Anti-RIP antibody [EPR4689] (ab125072)

All lanes : Anti-RIP antibody [EPR4689] (ab125072) at 1/1000 dilution (unpurified)

Lane 1: Raji cell lysate

Lane 2: Jurkat cell lysate

Lane 3: HeLa cell lysate

Lane 4: 293T cell lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 75 kDa



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- · Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

· Guarantee only valid for products bought direct from Abcam or one of our authorized distributors