abcam

Product datasheet

# Anti-RIP antibody [EPR24883-85] (BSA and Azide free) ab300618

KO VALIDATED Recombinant RabMAb

9 Images

## Overview

| | |
|---|---|
| **Product name** | Anti-RIP antibody [EPR24883-85] (BSA and Azide free) |
| **Description** | Rabbit monoclonal [EPR24883-85] to RIP - BSA and Azide free |
| **Host species** | Rabbit |
| **Specificity** | Not suitable for mouse and rat IHC-P. |
| **Tested applications** | **Suitable for:** WB, IHC-P, IP<br>**Unsuitable for:** Flow Cyt (Intra) or ICC/IF |
| **Species reactivity** | **Reacts with:** Mouse, Rat, Human |
| **Immunogen** | Recombinant fragment. This information is proprietary to Abcam and/or its suppliers. |
| **Positive control** | 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. |
| **General notes** | ab300618 is the carrier-free version of **ab300617**. |

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.

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**.

## Properties

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|---|---|
| **Form** | Liquid |
| **Storage instructions** | Shipped at 4°C. Store at +4°C. |
| **Storage buffer** | pH: 7.20<br>Constituent: 100% PBS |
| **Carrier free** | Yes |
| **Purity** | Protein A purified |
| **Clonality** | Monoclonal |
| **Clone number** | EPR24883-85 |
| **Isotype** | IgG |

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab300618 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** | | Use at an assay dependent concentration. Detects a band of approximately 75 kDa (predicted molecular weight: 75 kDa). |
| **IHC-P** | | Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| **IP** | | Use at an assay dependent concentration. |

**Application notes** Is unsuitable for Flow Cyt (Intra) or ICC/IF.

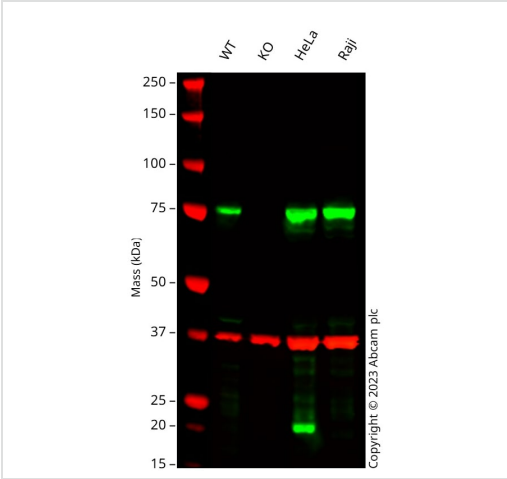
## Target

**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.<br>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.<br>Autophosphorylated on serine and threonine residues.<br>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 [EPR24883-85] (BSA and Azide free) (ab300618)

**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.

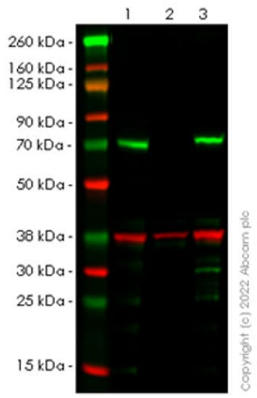
Performed under reducing conditions.

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

This data was developed using **ab300617**, the same antibody clone in a different buffer formulation.

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] (BSA and Azide free) (AB300618)

**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

This data was developed using **ab300617**, the same antibody clone in a different buffer formulation.

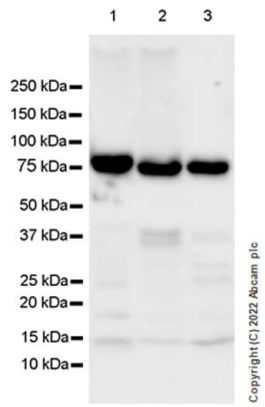
False colour image of Western blot: Anti-RIP antibody [EPR24883-85] (**ab300617**) staining at 1/1000 dilution, shown in green; Mbuse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red.

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] (BSA and Azide free) (AB300618)

**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

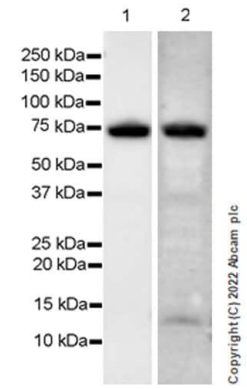
**Predicted band size:** 75 kDa
**Observed band size:** 75 kDa

**Exposure time:** 15 seconds

This data was developed using **ab300617**, the same antibody clone in a different buffer formulation.

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] (BSA and Azide free) (AB300618)

**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

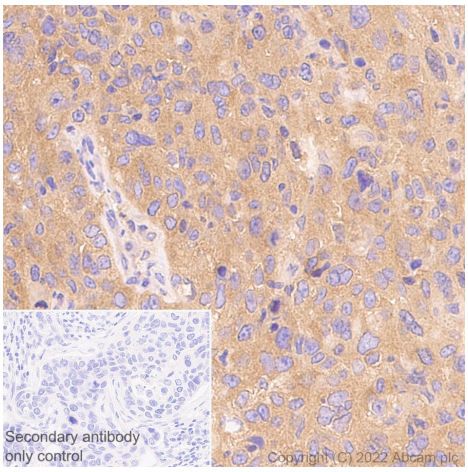
**Observed band size:** 75 kDa

This data was developed using **ab300617**, the same antibody clone in a different buffer formulation.

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

Exposure time:

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



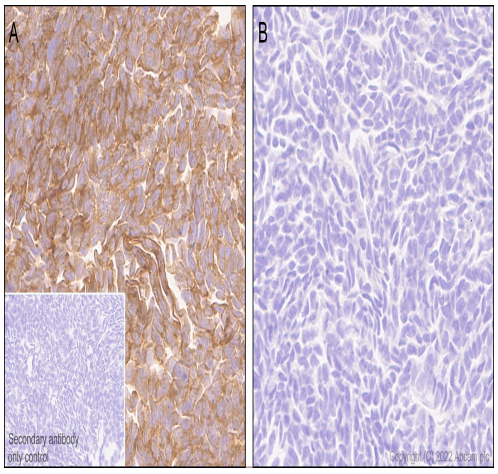

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RIP antibody [EPR24883-85] (BSA and Azide free) (AB300618)

This data was developed using **ab300617**, the same antibody clone in a different buffer formulation.

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.



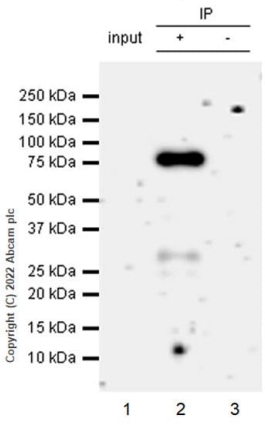

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RIP antibody [EPR24883-85] (BSA and Azide free) (AB300618)

This data was developed using **ab300617**, the same antibody clone in a different buffer formulation.

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] (BSA and Azide free) (AB300618)

This data was developed using **ab300617**, the same antibody clone in a different buffer formulation.

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.

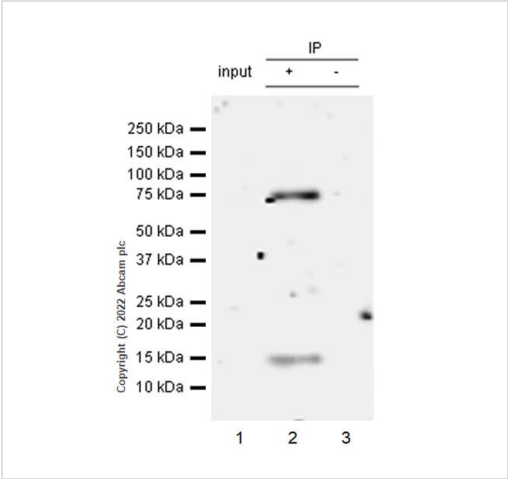
**Lane 1**: HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg

**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] (BSA and Azide free) (AB300618)

This data was developed using **ab300617**, the same antibody clone in a different buffer formulation.

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

**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] (BSA and Azide free) (AB300618)



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