

## Product datasheet

# Anti-RIP antibody [7H10] ab72139

**KO** **VALIDATED**

★★★★★ 2 Abreviews 7 References 4 Images

### Overview

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<b>Product name</b>	Anti-RIP antibody [7H10]
<b>Description</b>	Mouse monoclonal [7H10] to RIP
<b>Host species</b>	Mouse
<b>Tested applications</b>	<b>Suitable for:</b> ChIP, Flow Cyt, WB, IP, IHC-P
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human
<b>Immunogen</b>	Recombinant full length human RIP protein purified from E.coli (His/ABD-RIP)
<b>Positive control</b>	HeLa, K562 and SK-N-MC cell lysates
<b>General notes</b>	This product was changed from ascites to tissue culture supernatant on 18th September 2017. Lot numbers higher than GR304252 will be from tissue culture supernatant. Please note that the dilutions may need to be adjusted accordingly.

### Properties

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<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
<b>Storage buffer</b>	Preservative: 0.03% Sodium azide Constituents: 0.01% BSA, 50% Glycerol, 0.87% Sodium chloride, HEPES
<b>Purity</b>	Protein G purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	7H10
<b>Isotype</b>	IgG2b
<b>Light chain type</b>	kappa

### Applications

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Our [Abpromise guarantee](#) covers the use of **ab72139** in the following tested applications.

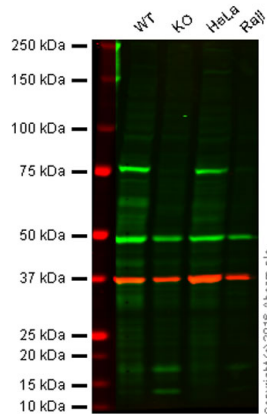
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ChIP		Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration. <a href="#">ab170192</a> - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.
WB	★★★★☆	Use a concentration of 1 µg/ml. Detects a band of approximately 76 kDa (predicted molecular weight: 76 kDa).
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

## Target

<b>Function</b>	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.
<b>Sequence similarities</b>	Belongs to the protein kinase superfamily. TKL Ser/Thr protein kinase family. Contains 1 death domain. Contains 1 protein kinase domain.
<b>Post-translational modifications</b>	Proteolytically cleaved by caspase-8 during TNF-induced apoptosis. Cleavage abolishes NF-kappa-B activation and enhances pro-apoptotic 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.
<b>Cellular localization</b>	Cytoplasm.

## Images



Western blot - Anti-RIP antibody [7H10] (ab72139)

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

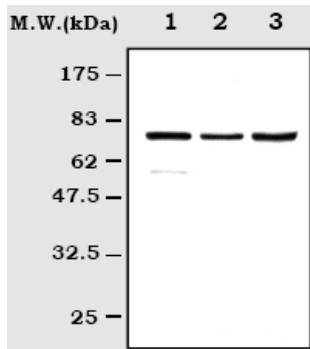
**Lane 2:** RIP knockout HAP1 cell lysate (20 µg)

**Lane 3:** HeLa cell lysate (20 µg)

**Lane 4:** Raji cell lysate (20 µg)

**Lanes 1 - 4:** Merged signal (red and green). Green - ab72139 observed at 78 kDa. Red - loading control, ab181602, observed at 37 kDa.

ab72139 was shown to recognize RIP in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when RIP knockout samples were examined. Wild-type and RIP knockout samples were subjected to SDS-PAGE. ab72139 at a dilution of 1/500 and ab181602 (loading control to GAPDH) at a dilution of 1/10,000 were incubated overnight at 4°C. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-RIP antibody [7H10] (ab72139)

**All lanes :** Anti-RIP antibody [7H10] (ab72139) at 1/500 dilution

**Lane 1 :** K562 cell lysate

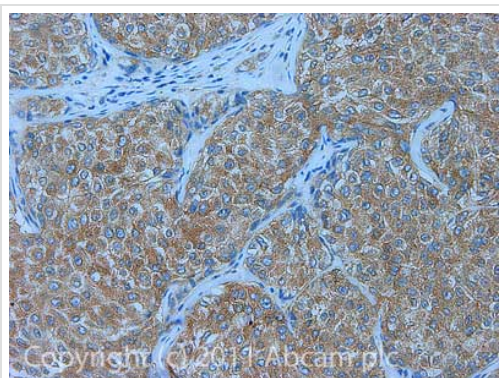
**Lane 2 :** HeLa cell lysate

**Lane 3 :** SK-N-MC cell lysate

**Predicted band size:** 76 kDa

**Observed band size:** 76 kDa

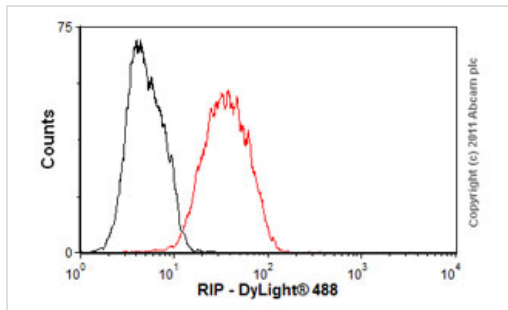
**Additional bands at:** 57 kDa. We are unsure as to the identity of these extra bands.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RIP antibody [7H10] (ab72139)

IHC image of ab72139 staining in Breast carcinoma formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab72139, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Flow Cytometry - Anti-RIP antibody [7H10] (ab72139)

Overlay histogram showing HEK293 cells stained with ab72139 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab72139, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (ab91366, 2µg/1x10<sup>6</sup> cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HEK293 cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

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