abcam

ab325636

Anti-RIP3 antibody [RM1355]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Multiclonal
Immunogens	The exact immunogen used to generate this antibody is proprietary information.
Clone number	RM1355
Purification technique	Affinity purification Protein A
Concentration	0.5 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Species	Mouse

Dilution info	1/1000
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/500
Notes	-

Species	Mouse
Dilution info	1/500
Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Species	Mouse
Dilution info	1/30
Notes	-

Target data

See full target information RIPK3

Function	Serine/threonine-protein kinase that activates necroptosis and apoptosis, two parallel forms of cell death (PubMed:19524512, PubMed:19524513, PubMed:22265413, PubMed:22265414, PubMed:22421439, PubMed:29883609, PubMed:32657447). Necroptosis, a programmed cell death process in response to death-inducing TNF family members, is triggered by RIPK3 following activation by ZBP1 (PubMed:19524512, PubMed:19524513, PubMed:22265413, PubMed:22265414, PubMed:22421439, PubMed:29883609, PubMed:32298652). Activated RIPK3 forms a necrosis-inducing complex and mediates phosphorylation of MLKL, promoting MLKL localization to the plasma membrane and execution of

programmed necrosis characterized by calcium influx and plasma membrane damage (PubMed:19524512, PubMed:19524513, PubMed:22265413, PubMed:22265414, PubMed:22421439, PubMed:25316792, PubMed:29883609). In addition to TNF-induced necroptosis, necroptosis can also take place in the nucleus in response to orthomyxoviruses infection: following ZBP1 activation, which senses double-stranded Z-RNA structures, nuclear RIPK3 catalyzes phosphorylation and activation of MLKL, promoting disruption of the nuclear envelope and leakage of cellular DNA into the cytosol (By similarity). Also regulates apoptosis: apoptosis depends on RIPK1, FADD and CASP8, and is independent of MLKL and RIPK3 kinase activity (By similarity). Phosphorylates RIPK1: RIPK1 and RIPK3 undergo reciprocal auto- and trans-phosphorylation (PubMed:19524513). In some cell types, also able to restrict viral replication by promoting cell death-independent responses (By similarity). In response to Zika virus infection in neurons, promotes a cell death-independent pathway that restricts viral replication: together with ZBP1, promotes a death-independent transcriptional program that modifies the cellular metabolism via up-regulation expression of the enzyme ACOD1/IRG1 and production of the metabolite itaconate (By similarity). Itaconate inhibits the activity of succinate dehydrogenase, generating a metabolic state in neurons that suppresses replication of viral genomes (By similarity). RIPK3 binds to and enhances the activity of three metabolic enzymes: GLUL, GLUD1, and PYGL (PubMed:19498109). These metabolic enzymes may eventually stimulate the tricarboxylic acid cycle and oxidative phosphorylation, which could result in enhanced ROS production (PubMed:19498109). (Microbial infection) In case of herpes simplex virus 1/HHV-1 infection, forms heteromeric amyloid structures with HHV-1 protein RIR1/ICP6 which may inhibit RIPK3-mediated necroptosis, thereby preventing host cell death pathway and allowing viral evasion.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are recombinant multiclonals?
Recombinant multiclonals are a mixture of recombinant antibodies co-expressed from a library of heavy and light chains. They offer several advantages including:

- The sensitivity of polyclonal antibodies by recognising multiple epitopes
- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

View our range of recombinant multiclonal antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

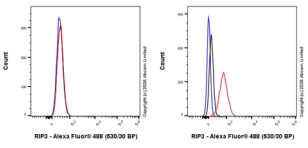
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

9 product images



Flow Cytometry (Intracellular) - Anti-RIP3 antibody [RM1355] (ab325636)

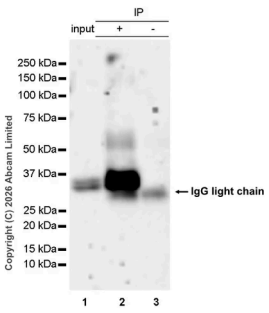
RIP3 Flow Cytometry (Intracellular) staining using rabbit Anti-RIP3 antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized THP-1 (human monocytic leukemia monocyte) (Right) HepG2 (human hepatocellular carcinoma epithelial cell) (Left) cells labelling RIP3 with ab325636 at 1/500 dilution (0.1ug) / Red compared

with a Rabbit monoclonal IgG (ab172730) / Black isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.

Negative control: HepG2.



Immunoprecipitation - Anti-RIP3 antibody [RM1355] (ab325636)

RIP3 was immunoprecipitated from 0.35 mg HT-29 (human colorectal adenocarcinoma epithelial cell) whole cell lysate with ab325636 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab325636 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: HT-29 (human colorectal adenocarcinoma epithelial cell) whole cell lysate
Lane 2: ab325636 IP in HT-29 (human colorectal adenocarcinoma epithelial cell) whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab325636 in HT-29 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 180 seconds.

All lanes:
Immunoprecipitation - Anti-RIP3 antibody [RM1355] (ab325636) at 1/1000 dilution

Lane 1:
HT-29 (human colorectal adenocarcinoma epithelial cell) whole cell lysate at 10 µg

Lane 2:
ab325636 at 1/30 IP in HT-29 (human colorectal adenocarcinoma epithelial cell) whole cell lysate

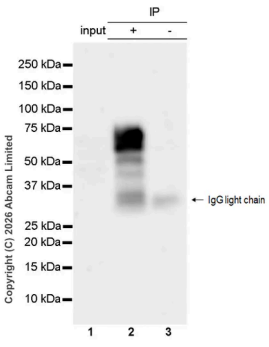
Lane 3:
Rabbit monoclonal IgG (ab172730) instead of ab325636 in HT-29 whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Observed band size: 37-56 kDa

Exposure time: 180s



Immunoprecipitation - Anti-RIP3 antibody [RM1355] (ab325636)

RIP3 was immunoprecipitated from 0.35 mg THP-1 (human monocytic leukemia monocyte) whole cell lysate with ab325636 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab325636 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: THP-1 (human monocytic leukemia monocyte) whole cell lysate at
Lane 2: ab325636 IP in THP-1 (human monocytic leukemia monocyte) whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab316957 in THP-1 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 50 seconds.

To minimize protein degradation, cells were lysed immediately after harvest and then applied for Immunoprecipitation as soon as possible.
All lanes:
Immunoprecipitation - Anti-RIP3 antibody [RM1355] (ab325636) at 1/1000 dilution

Lane 1:
THP-1 (human monocytic leukemia monocyte) whole cell lysate at at 10 µg

Lane 2:
ab325636 at 1/30 IP in THP-1 (human monocytic leukemia monocyte) whole cell lysate

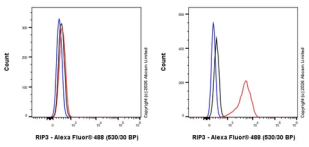
Lane 3:
Rabbit monoclonal IgG (ab172730) instead of ab316957 in THP-1 whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Observed band size: 37-60 kDa

Exposure time: 50s



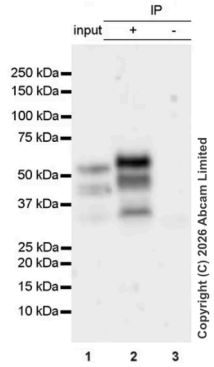
Flow Cytometry (Intracellular) - Anti-RIP3 antibody [RM1355] (ab325636)

RIP3 Flow Cytometry (Intracellular) staining using rabbit Anti-RIP3 antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized L-929 (mouse connective tissue fibroblast) (Right) Neuro-2a (mouse neuroblastoma neuroblast) (Left) cells labelling RIP3 with ab325636 at 1/500 dilution (0.1ug) / Red compared with a Rabbit monoclonal IgG (ab172730) / Black isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.

Negative control: Neuro-2a.



Immunoprecipitation - Anti-RIP3 antibody [RM1355] (ab325636)

RIP3 was immunoprecipitated from 0.35 mg L-929 (mouse connective tissue fibroblast) whole cell lysate with ab325636 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab325636 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (ab131366) was used at 1/5000 dilution.

Lane 1: L-929 (mouse connective tissue fibroblast) whole cell lysate
Lane 2: ab325636 IP in L-929 (mouse connective tissue fibroblast) whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab325636 in L-929 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 32 seconds.

All lanes:
Immunoprecipitation - Anti-RIP3 antibody [RM1355] (ab325636) at 1/1000 dilution

Lane 1:

L-929 (mouse connective tissue fibroblast) whole cell lysate at 10 µg

Lane 2:
ab325636 at 1/30 IP in L-929 (mouse connective tissue fibroblast) whole cell lysate

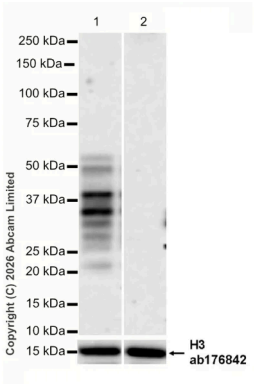
Lane 3:
Rabbit monoclonal IgG (ab172730) instead of ab325636 in L-929 whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Observed band size: 37-56 kDa

Exposure time: 32s



Western blot - Anti-RIP3 antibody [RM1355] (ab325636)

RIP3 Western blot staining using rabbit Anti-RIP3 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Performed under reducing conditions.

In Western blot, ab325636 was shown to bind specifically to RIPK3. Target of interest was observed at 37-50 kDa in wild-type THP-1 cell lysates (lane 1) with no signal observed at this size in RIPK3 knockout cell line (lane 2)(knockout cell line ab280065).

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) (1:100000) (17KDa).

All lanes:
Western blot - Anti-RIP3 antibody [RM1355] (ab325636) at 1/1000 dilution

Lane 1:
Wilde-type THP-1 (human monocytic leukemia monocyte) whole cell lysate at 20 µg

Lane 2:
Western blot - Human RIPK3 knockout THP-1 cell line (ab280065) at 20 µg

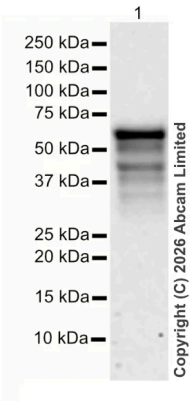
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 37-56 kDa, 17 kDa

Exposure time: 180s



Western blot - Anti-RIP3 antibody [RM1355] (ab325636)

RIP3 Western blot staining of THP-1 (human monocytic leukemia monocyte) whole cell lysate using rabbit Anti-RIP3 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

To minimize protein degradation, cells/tissues were lysed immediately after harvest and then applied to a gel and transfer membrane for Western blotting as soon as possible.

The bands beneath the target band (56 kDa) are likey to be degradation products.

This blot was developed using a high-sensitivity ECL substrate, allowing for the detection of proteins in the mid-femtogram range.

All lanes:
Western blot - Anti-RIP3 antibody [RM1355] (ab325636) at 1/1000 dilution

All lanes:
THP-1 (human monocytic leukemia monocyte) whole cell lysate at 20 µg

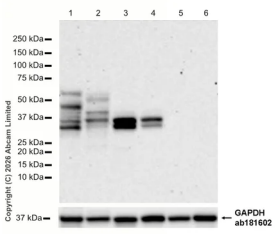
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Observed band size: 37-56 kDa

Exposure time: 180s



Western blot - Anti-RIP3 antibody [RM1355] (ab325636)

RIP3 Western blot staining using rabbit Anti-RIP3 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Negative control: HeLa (PMID: 26269957, PMID: 28179994), HepG2 (PMID: 25675296).

The bands beneath the target band (56 kDa) are likey to be degradation products.

This blot was developed using a high-sensitivity ECL substrate, allowing for the detection of proteins in the mid-femtogram range.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) (1:200000) (36KDa).

All lanes:
Western blot - Anti-RIP3 antibody [RM1355] (ab325636) at 1/1000 dilution

Lane 1:
COLO 205 (human colon adenocarcinoma tumor ascitic epithelial cell) whole cell lysate at 20 µg

Lane 2:
THP-1 (human monocytic leukemia monocyte) whole cell lysate at 20 µg

Lane 3:
HT-29 (human colorectal adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 4:
Jurkat (human T cell leukemia T lymphocyte from peripheral blood) whole cell lysate at 20 µg

Lane 5:
HepG2 (human hepatocellular carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 6:
HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 20 µg

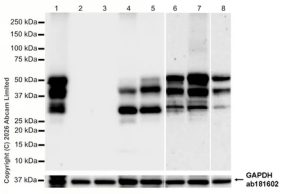
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Observed band size: 37-56 kDa, 36 kDa

Exposure time: 180s



Western blot - Anti-RIP3 antibody [RM1355] (ab325636)

RIP3 Western blot staining using rabbit Anti-RIP3 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Negative control: Neuro-2a, B16-F10 (PMID: 25858093).

The bands beneath the target band (56 kDa) are likey to be degradation products.

This blot was developed using a high-sensitivity ECL substrate, allowing for the detection of proteins in the mid-femtogram range.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) (1:200000) (36KDa).

All lanes:
Western blot - Anti-RIP3 antibody [RM1355] (ab325636) at 1/1000 dilution

Lane 1:
L-929 (mouse connective tissue fibroblast) whole cell lysate at 20 µg

Lane 2:
Neuro-2a (mouse neuroblastoma neuroblast) whole cell lysate at 20 µg

Lane 3:
B16-F10 (mouse skin melanoma cell) whole cell lysate at 20 µg

Lane 4:
J774A.1 (mouse reticulum cell sarcoma monocyte/macrophage) whole cell lysate at 20 µg

Lane 5:
RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Lane 6:
WEHI-231 (mouse B cell lymphoma B lymphocyte) whole cell lysate at 20 µg

Lane 7:
Mouse colon tissue lysate at 20 µg

Lane 8:
Mouse testis tissue lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Observed band size: 37-56 kDa, 36 kDa

Exposure time: 180s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.