Anti-Phospho-RIP3 (S232) Antibody [JE44-62] HA721428

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Mouse, Rat
Applications:	WB, IF-Cell
Molecular Wt:	Predicted band size: 53 kDa
Clone number:	JE44-62

Description: Serine/threonine-protein kinase that activates necroptosis and apoptosis, two parallel forms of cell death. Necroptosis, a programmed cell death process in response to death-inducing TNF-alpha family members, is triggered by RIPK3 following activation by ZBP1. 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. 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. Also regulates apoptosis: apoptosis depends on RIPK1, FADD and CASP8, and is independent of MLKL and RIPK3 kinase activity. Phosphorylates RIPK1: RIPK1 and RIPK3 undergo reciprocal auto- and trans-phosphorylation (By similarity). In some cell types, also able to restrict viral replication by promoting cell death-independent responses. In response to flavivirus 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. Itaconate inhibits the activity of succinate dehydrogenase, generating a metabolic state in neurons that suppresses replication of viral genomes. RIPK3 binds to and enhances the activity of three metabolic enzymes: GLUL, GLUD1, and PYGL (By similarity). These metabolic enzymes may eventually stimulate the tricarboxylic acid cycle and oxidative phosphorylation, which could result in enhanced ROS production (By similarity).

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser232 of Human RIP3.

Positive control: L-929 treated with 20μM Z-VAD for 3.5 hours add 100nM SM-164 and 20ng/ml TNF-α for 3 hours whole cell lysate, L-929 cells treated with 20μM Z-VAD for 2.75 hours, add 100nM SM-164 and 20ng/ml TNF-α for 2.25 hours.

Subcellular location: Cytoplasm, cytosol, Nucleus.

Database links: SwissProt: Q9QZL0 Mouse | Q9Z2P5 Rat

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:100

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

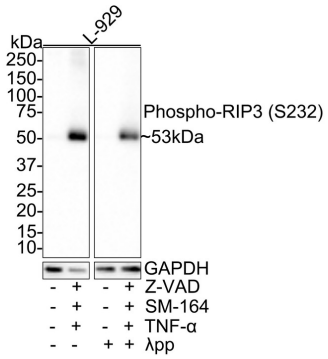

Fig1: Western blot analysis of Phospho-RIP3 (S232) on different lysates with Rabbit anti-Phospho-RIP3 (S232) antibody (HA721428) at 1/1,000 dilution.

Lane 1: L-929 whole cell lysate
Lane 2: L-929 treated with 20μM Z-VAD for 3.5 hours, add 100nM SM-164 and 20ng/ml TNF-α for 3 hours whole cell lysate
Lane 3: L-929 treated with λpp for 1 hour whole cell lysate
Lane 4: L-929 treated with 20μM Z-VAD for 3.5 hours, add 100nM SM-164 and 20ng/ml TNF-α for 3 hours, treated with λpp for 1 hour whole cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 53 kDa
Observed band size: 53 kDa

Exposure time: 20 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721428) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

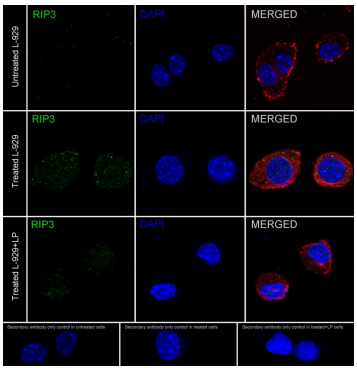

Fig2: Immunocytochemistry analysis of L-929 cells treated with or without 20μM Z-VAD for 2.75 hours, add 100nM SM-164 and 20ng/ml TNF-α for 2.25 hours, then treated with λpp for 1 hour labeling Phospho-RIP3 (S232) with Rabbit anti-Phospho-RIP3 (S232) antibody (HA721428) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Phospho-RIP3 (S232) antibody (HA721428) at 1/100 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Zeng X et al. Activated Drp1 regulates p62-mediated autophagic flux and aggravates inflammation in cerebral ischemia-reperfusion via the ROS-RIP1/RIP3-exosome axis. Mil Med Res. 2022 May
2. Jeon J et al. TRIM24-RIP3 axis perturbation accelerates osteoarthritis pathogenesis. Ann Rheum Dis. 2020 Dec