

# Product Datasheet

## RIPK3/RIP3 Antibody - BSA Free NBP1-77299

Unit Size: 0.1 mg

Store at 4C.

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**NBP1-77299**

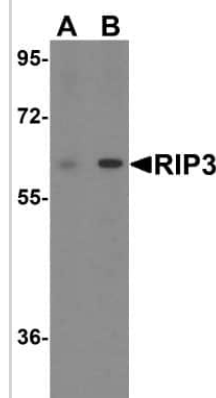
RIPK3/RIP3 Antibody - BSA Free

Product Information	
Unit Size	0.1 mg
Concentration	1 mg/ml
Storage	Store at 4C.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Peptide affinity purified
Buffer	PBS
Target Molecular Weight	53 kDa
Product Description	
Host	Rabbit
Gene ID	11035
Gene Symbol	RIPK3
Species	Human, Mouse, Rat
Reactivity Notes	Human, mouse and rat reactivity reported in multiple pieces of scientific literature.
Specificity/Sensitivity	Mouse RIPK3/RIP3 Antibody has one isoform (486aa, 53 kDa). Human RIP3 has 3 isoforms, including isoform 1 (518aa, 57 kDa), isoform 2 (252aa, 28 kDa) and isoform 3 (231aa, 25 kDa). Rat RIP3 also has one isoform (478aa, 52 kDa). NBP1-77299 can detect can detect isoforms of mouse and rat as well as human isoform 1.
Immunogen	RIPK3/RIP3 Antibody was made to a 14 amino acid peptide near the carboxy terminus of murine RIP3. The immunogen is located within the last 50 amino acids of RIP3. Amino Acid Squence: AQFGRGRGWQPFHK
Product Application Details	
Applications	Western Blot, ELISA, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Gel Supershift Assay, Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 0.1-0.5 ug/ml, ELISA 1:100 - 1:2000, Immunohistochemistry 5 ug/ml, Immunocytochemistry/ Immunofluorescence 20 ug/ml, Immunoprecipitation 20 ug/mL, Immunohistochemistry-Paraffin 5 ug/ml, Gel Supershift Assay, Knockout Validated, Knockdown Validated
Application Notes	Gel supershift assay reported in scientific literature [PMID: 27721066]. Immunoprecipitation reported in scientific literature [PMID: 27861127]. RIPK3/RIP3 antibody validated for WB from a verified customer review. Knockout validation reported in scientific literature [PMID: 32246911]. Knockdown validation reported in scientific literature [PMID: 31655343]. ICC/IF, IHC, and WB reported in multiple pieces of scientific literature.

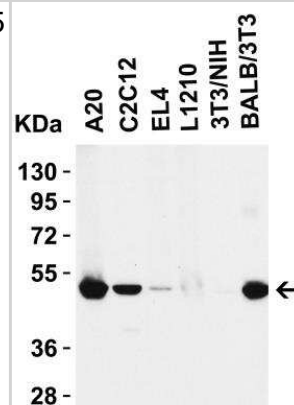


## Images

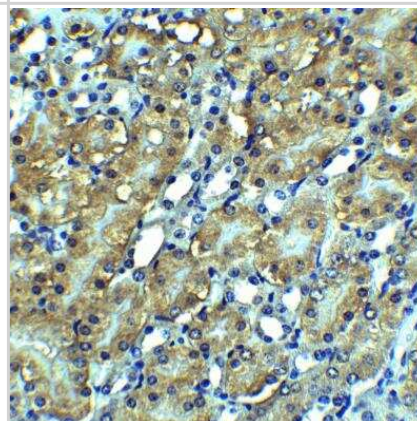
Western Blot: RIPK3/RIP3 Antibody [NBP1-77299] - Analysis of RIP3 in HeLa cell lysate with RIPK3/RIP3 antibody [NBP1-77299] at (A) 1 and (B) 2 ug/mL. Observed molecular weight ~60 kDa. Theoretical molecular weight 53 kDa.



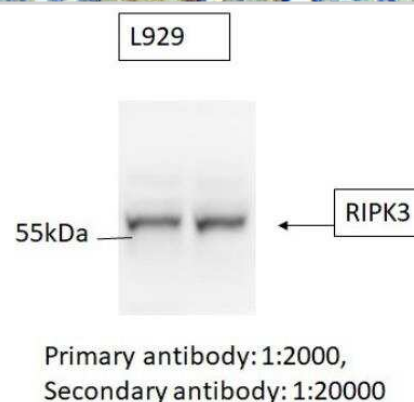
Western Blot: RIPK3/RIP3 Antibody [NBP1-77299] - Mouse Cell lines. 15 ug of lysates per lane. Antibodies: [NBP1-77299], (0.5 ug/mL), 1h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution. Observed molecular weight ~50 kDa. Theoretical molecular weight 53 kDa.



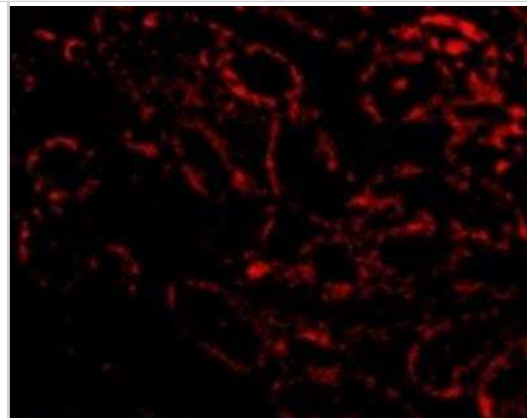
Immunohistochemistry-Paraffin: RIPK3/RIP3 Antibody [NBP1-77299] - Immunohistochemistry analysis of a paraffin embedded mouse kidney tissue section using RIPK3/RIP3 Antibody [NBP1-77299] at 2.5 ug/mL concentration.



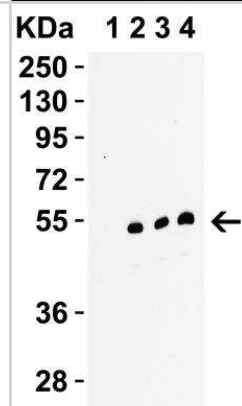
Western Blot: RIPK3/RIP3 Antibody [NBP1-77299] - Analysis using RIPK3/RIP3 antibody [NBP1-77299]. L929 cell line (mouse). 20 ug whole cell lysate. Primary antibody: 1:2000. Secondary antibody: 1:20000. Western blot image submitted by a verified customer review. Observed molecular weight ~57 kDa. Theoretical molecular weight 53 kDa.



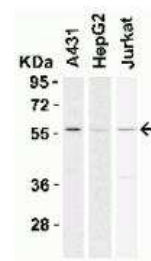
Immunocytochemistry/Immunofluorescence: RIPK3/RIP3 Antibody [NBP1-77299] - ICC/IF analysis of Rat's kidney cell culture using RIPK3/RIP3 Antibody [NBP1-77299] at 20 ug/mL concentration.



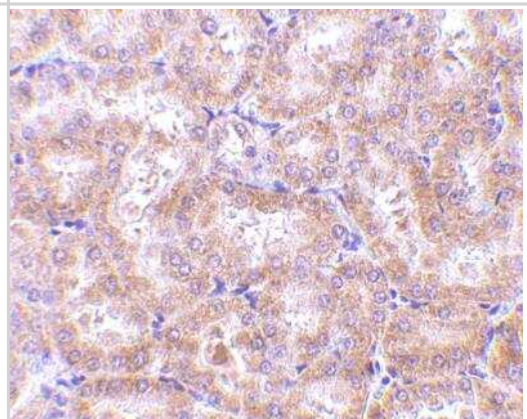
Western Blot: RIPK3/RIP3 Antibody [NBP1-77299] - C2C12 Cells. 15 ug of lysates per lane. Antibodies: RIPK3/RIP3 antibody [NBP1-77299], 1h incubation at RT in 5% NFD/MTBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution. Lane 1: NBP1-77299, 0.1 ug/mL in the presence of peptide blocking. Lane 2: NBP1-77299, 0.1 ug/mL Lane 3: NBP1-77299, 0.2 ug/mL Lane 4: NBP1-77299, 0.5 ug/mL. Observed molecular weight ~55 kDa. Theoretical molecular weight 53 kDa.



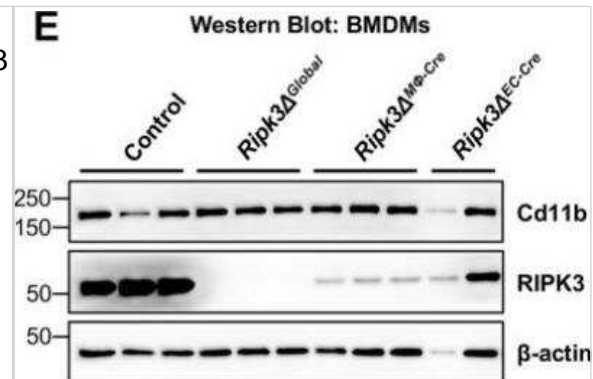
Western Blot: RIPK3/RIP3 Antibody [NBP1-77299] - Human Cell Lines. 15 ug of lysates per lane. Antibodies: RIPK3/RIP3 antibody [NBP1-77299], (0.5 ug/mL), 1h incubation at RT in 5% NFD/MTBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution. Observed molecular weight ~ 57 kDa. Theoretical molecular weight 53 kDa.



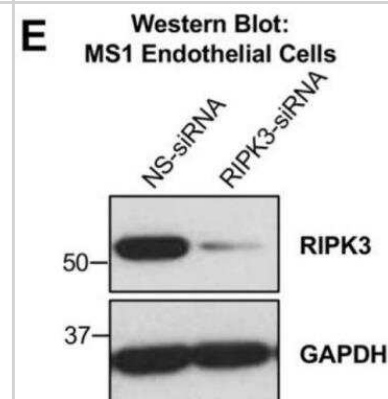
Immunohistochemistry-Paraffin: RIPK3/RIP3 Antibody [NBP1-77299] - Rat kidney tissue with RIPK3/RIP3 Antibody [NBP1-77299] at 5 ug/mL.



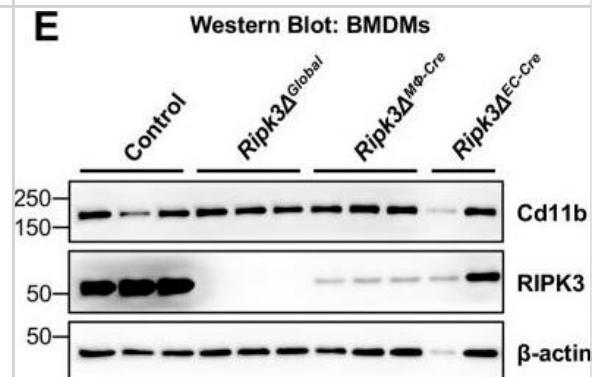
Knockout Validated: RIPK3/RIP3 Antibody [NBP1-77299] - Bone-marrow-derived macrophages exhibit significant reduction in RIPK3/RIP3 levels in RIPK3/RIP3deltaGlobal, RIPK3/RIP3deltaMphi-Cre and RIPK3/RIP3deltaEC-Cre mice. Protein lysates were immunoblotted to identify CD11b, RIPK3/RIP3 and beta-actin (loading control). Image collected and cropped by CiteAb from the following publication ([www.dmm.biologists.org/lookup/doi/10.1242/dmm.041962](http://www.dmm.biologists.org/lookup/doi/10.1242/dmm.041962)) licensed under a CC-BY license.



Knockdown Validated: RIPK3/RIP3 Antibody [NBP1-77299] - RIPK3/RIP3-deficiency impacts MCP-1 and IL-1beta expression in BMDMs and MCP-1 and E-Selectin/CD62E expression in cultured endothelial cells. Protein lysates were immunoblotted for RIPK3/RIP3 and the loading control, GAPDH (n=4 independent experiments). Image collected and cropped by CiteAb from the following publication ([www.dmm.biologists.org/content/13/1/dmm041962](http://www.dmm.biologists.org/content/13/1/dmm041962)) licensed under a CC-BY license.

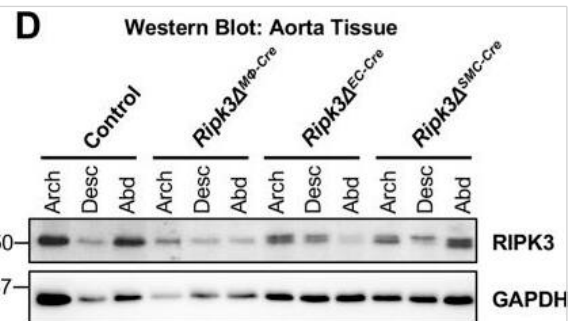


Western Blot: RIPK3/RIP3 Antibody - BSA Free [NBP1-77299] - Bone-marrow-derived macrophages exhibit significant reduction in RIPK3 levels in Ripk3ΔGlobal, Ripk3ΔMφ-Cre & Ripk3ΔEC-Cre mice. (A,B) BMDMs were isolated, differentiated on chamber slides for 7 days, immunostained for macrophage markers CD68 (red; A) & CD11b (pink; B), & co-stained for nuclei (Hoechst; blue). All cells from seven separate BMDM isolations were positive for these markers (n=7). (C) A phagocytosis assay was performed on BMDMs with fluorescent polystyrene beads. Phagocytic cells display TRITC+/Hoechst+ signal, & 85% of cells from two separate BMDM isolations were double positive (n=2). (D-F) Protein or RNA was collected from control, Ripk3ΔGlobal, Ripk3ΔMφ-Cre & Ripk3ΔEC-Cre BMDMs. RNA was converted to cDNA & analyzed by qPCR for Ripk3 levels (D). Protein lysates were immunoblotted to identify CD11b, RIPK3 & β-actin (loading control) (E) & quantified (F). (G) RNA from control & Ripk3ΔGlobal BMDMs was converted to cDNA & analyzed by qPCR for Ripk3-Exon 10 levels. For panels D,F,G, each dot represents a BMDM isolation from an individual animal. Statistics for panels D & F were calculated using one-way ANOVA with Dunnett's multiple comparisons test. Overall ANOVA P-values (prior to the post hoc tests) are 0.0002 (D) & <0.0001 (F). Statistics for panel G were calculated using an unpaired t-test with Welch's correction. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001. Data are mean±s.d. Scale bars: 25 μm. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31953345>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.





Western Blot: RIPK3/RIP3 Antibody - BSA Free [NBP1-77299] - p-MLKL levels are nearly undetectable in advanced plaques. (A-C) After 3 months on a Western diet, protein was collected from control (n=7), Ripk3 $\Delta$ M $\Phi$ -Cre (n=5), Ripk3 $\Delta$ EC-Cre (n=3) & Ripk3 $\Delta$ SMC-Cre (n=3) aortas. Protein lysates were immunoblotted to identify p-MLKL (Abcam; #ab196436), MLKL &  $\beta$ -actin (loading control) (A) & quantified (B,C). A faint positive p-MLKL signal can be seen in the representative blot in the control arch region; however, we could only detect p-MLKL in two out of the 18 aortas analyzed (B). Note that the same transfer membrane used for detecting CD11b (in Fig. 4S) was reprobed for p-MLKL & MLKL in A; the  $\beta$ -actin control blots are therefore the same in both figures. (D,E) Protein lysates were immunoblotted to identify RIPK3 & GAPDH (loading control) (D) & quantified (E). For panels B,C,E, each dot represents an individual animal. Statistics were calculated using two-way ANOVA. Overall ANOVA P-values are 0.76 (B), 0.07 (C) & 0.46 (E). Data are mean $\pm$ s.d. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31953345>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Rudalska R, Harbig J, Snaebjornsson M et al. LXR alpha activation and Raf inhibition trigger lethal lipotoxicity in liver cancer *Nature Cancer* 2021-02-01 [PMID: 35122079]

Wu Z, Fournel L, Stadler N et al. Modulation of lung cancer cell plasticity and heterogeneity with the restoration of cisplatin sensitivity by Neurotensin antibody *Cancer Lett.* 2018-12-21 [PMID: 30583074]

Jia Y, Cheng L, Yang J et al. miR-223-3p Prevents Necroptotic Macrophage Death by Targeting Ripk3 in a Negative Feedback Loop and Consequently Ameliorates Advanced Atherosclerosis *Arteriosclerosis, thrombosis, and vascular biology* 2023-11-16 [PMID: 37970714]

Selvarani R, Van Michelle Nguyen H, Thadathil N et al. Characterization of novel mouse models to study the role of necroptosis in aging and age-related diseases *GeroScience* 2023-10-04 [PMID: 37792157] (Western Blot, Mouse)

Taraborrelli L, ?enbabao?lu Y, Wang L et al. Tumor-intrinsic expression of the autophagy gene Atg16l1 suppresses anti-tumor immunity in colorectal cancer *Nat Commun* 2023-09-23 [PMID: 37741832] (Western Blot)

Zhou H, Liu L, Ma X et al. RIP1/RIP3/MLKL-mediated necroptosis contributes to vinblastine-induced myocardial damage *Molecular and Cellular Biochemistry* 2021-02-01 [PMID: 33247805]

Zhou H, Zhou L, Guan Q et al. Skp2-mediated MLKL degradation confers cisplatin-resistant in non-small cell lung cancer cells *Communications Biology* 2023-08-02 [PMID: 37532777]

Yan WT, Zhao WJ, Hu XM et al. PANoptosis-like cell death in ischemia/reperfusion injury of retinal neurons *Neural Regen Res* 2022-07-28 [PMID: 35900430]

Lee J, Lee S, Min S, Kang SW. RIP3-Dependent Accumulation of Mitochondrial Superoxide Anions in TNF- $\alpha$ -Induced Necroptosis *Molecules and Cells* 2022-04-30 [PMID: 35289306] (Block/Neutralize)

Thadathil N, Nicklas EH, Mohammed S et al. Necroptosis increases with age in the brain and contributes to age-related neuroinflammation *GeroScience* 2021-10-01 [PMID: 34515928] (Block/Neutralize)

Wen H, Chen J, Zhao B et al. Repression of ferroptotic cell death by mitochondrial calcium signaling *Research square* 2023-07-10 [PMID: 37502961] (IP, WB, Human)

Mohammed S, Thadathil N, Ohene-Marfo P et al. Absence of either Ripk3 or Mlkl reduces incidence of hepatocellular carcinoma independent of liver fibrosis *Molecular cancer research : MCR* 2023-05-19 [PMID: 37204757] (WB, Mouse)

More publications at <http://www.novusbio.com/NBP1-77299>



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General: novus@novusbio.com

### **Products Related to NBP1-77299**

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NBP1-77299PEP	RIPK3/RIP3 Antibody Blocking Peptide
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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### **Limitations**

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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