

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	
Description	Novus Biologicals Knockout (KO) Validated Rabbit RIPK3/RIP3 Antibody - BSA Free (NBP1-77299) is a polyclonal antibody validated for use in IHC, WB, ELISA, ICC/IF, Simple Western and IP. Anti-RIPK3/RIP3 Antibody: Cited in 81 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
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 Sequence: AQFGRGRGWQPFHK
Product Application Details	
Applications	Western Blot, Simple Western, Immunohistochemistry-Paraffin, ELISA, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunoprecipitation, Gel Supershift Assay, Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 0.1-0.5 ug/ml, Simple Western 1:25, 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

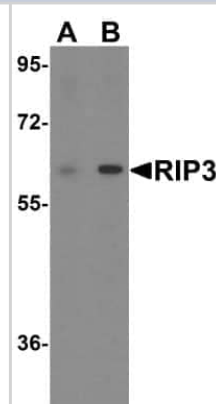


Application Notes

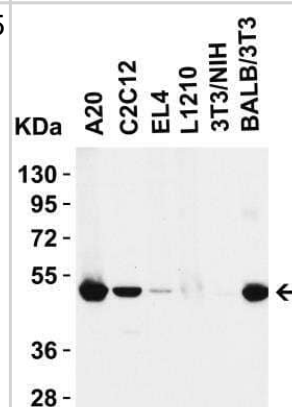
Gel supershift assay reported in scientific literature [PMID: 27721066]. Immunoprecipitation reported in scientific literature [PMID: 27861127]. RIPK3/RIP3 antibody validated for WB from a verified customer review. Knockout validation reported in scientific literature [PMID: 32246911]. Knockdown validation reported in scientific literature [PMID: 31655343]. ICC/IF, IHC, and WB reported in multiple pieces of scientific literature. See [Simple Western Antibody Database](#) for Simple Western validation: Tested in Mouse, rat heart tissue 0.25 mg/mL, separated by Size, antibody dilution of 1:25, apparent MW was 70 kDa

Images

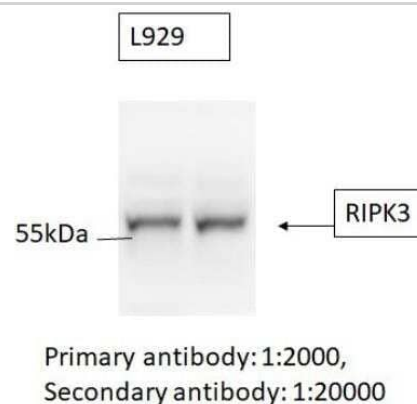
Western Blot: RIPK3/RIP3 Antibody [NBP1-77299] - Analysis of RIP3 in HeLa cell lysate with RIPK3/RIP3 antibody [NBP1-77299] at (A) 1 and (B) 2 ug/mL. Observed molecular weight ~60 kDa. Theoretical molecular weight 53 kDa.



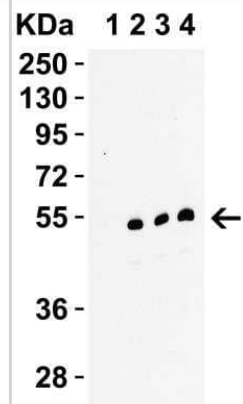
Western Blot: RIPK3/RIP3 Antibody [NBP1-77299] - Mouse Cell lines. 15 ug of lysates per lane. Antibodies: [NBP1-77299], (0.5 ug/mL), 1h incubation at RT in 5% NFDN/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution. Observed molecular weight ~50 kDa. Theoretical molecular weight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



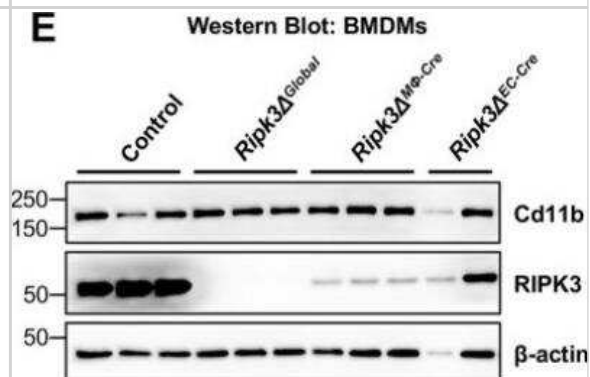
Western Blot: RIPK3/RIP3 Antibody [NBP1-77299] - Analysis using RIPK3/RIP3 antibody [NBP1-77299]. L929 cell line (mouse). 20 ug whole cell lysate. Primary antibody: 1:2000. Secondary antibody: 1:20000. Western blot image submitted by a verified customer review. Observed molecular weight ~57 kDa. Theoretical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



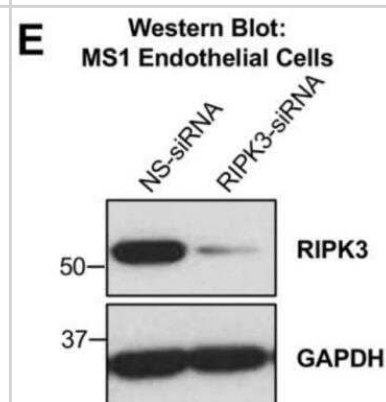
Western Blot: RIPK3/RIP3 Antibody [NBP1-77299] - C2C12 Cells. 15 ug of lysates per lane. Antibodies: RIPK3/RIP3 antibody [NBP1-77299], 1h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution. Lane 1: NBP1-77299, 0.1 ug/mL in the presence of peptide blocking. 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.



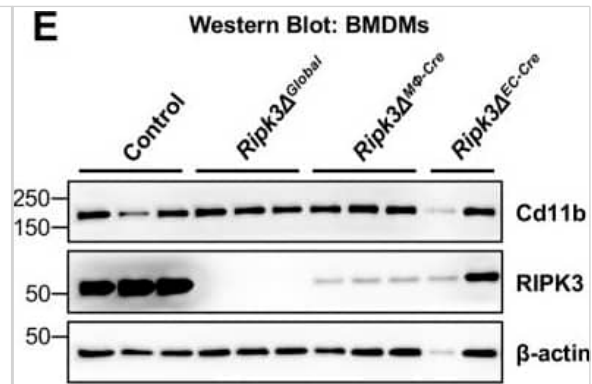
RIPK3-RIP3-Antibody-Knockout-Validated-NBP1-77299-img0025.jpg



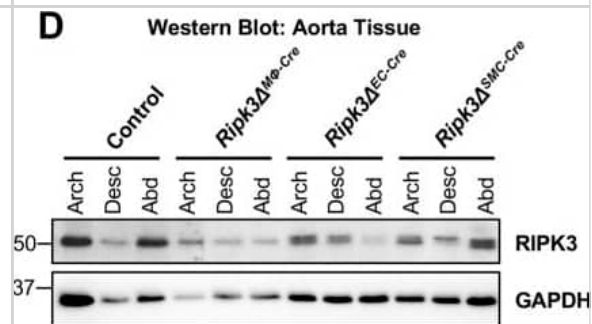
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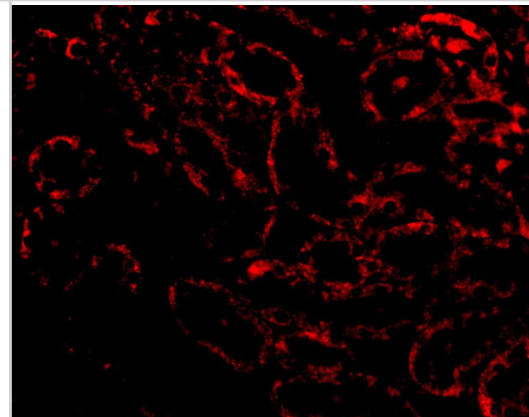
Western Blot: RIPK3/RIP3 Antibody - BSA Free [NBP1-77299] - Bone-marrow-derived macrophages exhibit significant reduction in RIPK3 levels in *Ripk3ΔGlobal*, *Ripk3ΔMΦ-Cre* & *Ripk3ΔEC-Cre* mice. (A,B) BMDMs were isolated, differentiated on chamber slides for 7 days, immunostained for macrophage markers 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 \pm s.d. Scale bars: 25 μ m. 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.



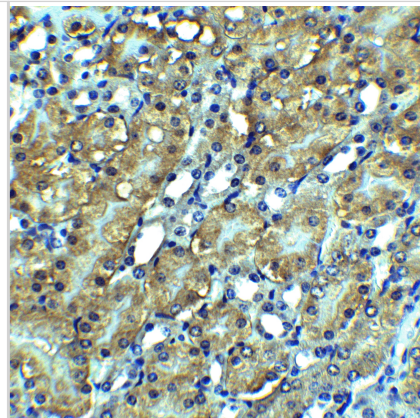
Western Blot: RIPK3/RIP3 Antibody - BSA Free [NBP1-77299] - p-MLKL levels are nearly undetectable in advanced plaques. (A-C) After 3 months on a Western diet, protein was collected from control (n=7), *Ripk3ΔMΦ-Cre* (n=5), *Ripk3ΔEC-Cre* (n=3) & *Ripk3ΔSMC-Cre* (n=3) aortas. Protein lysates were immunoblotted to identify p-MLKL (Abcam; #ab196436), MLKL & β -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 β -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.



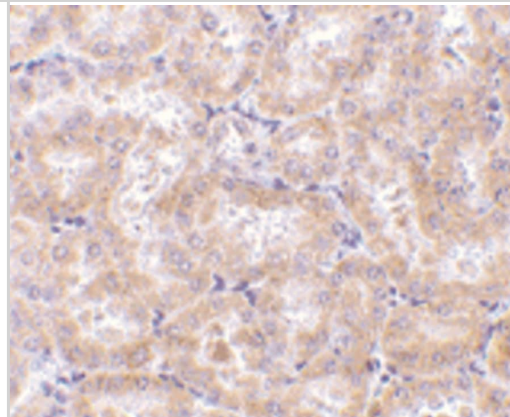
Immunocytochemistry/ Immunofluorescence: RIPK3/RIP3 Antibody - BSA Free [NBP1-77299] - Validation of RIPK3/RIP3 in Rat Kidney Tissue. Immunofluorescent analysis of 4% paraformaldehyde-fixed Rat Kidney tissue labeling RIPK3/RIP3 with at 20 μ g/mL, followed by goat anti-rabbit IgG secondary antibody at 1/500 dilution (red).



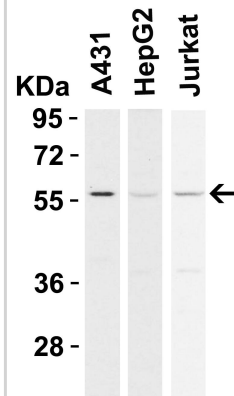
Immunohistochemistry: RIPK3/RIP3 Antibody - BSA Free [NBP1-77299]
 - Validation of RIPK3/RIP3 in Mouse Kidney Tissue. Immunohistochemical analysis of paraffin-embedded mouse kidney tissue using anti-RIPK3/RIP3 antibody at 2.5 ug/ml. Tissue was fixed with formaldehyde and blocked with 10% serum for 1 h at RT; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody overnight at 4C. A goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter stained with Hematoxylin.



Immunohistochemistry: RIPK3/RIP3 Antibody - BSA Free [NBP1-77299]
 - Validation of RIPK3/RIP3 in Rat Kidney Tissue. Immunohistochemical analysis of paraffin-embedded rat kidney tissue using anti-RIPK3/RIP3 antibody at 5 ug/ml. Tissue was fixed with formaldehyde and blocked with 10% serum for 1 h at RT; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody overnight at 4C. A goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter stained with Hematoxylin.

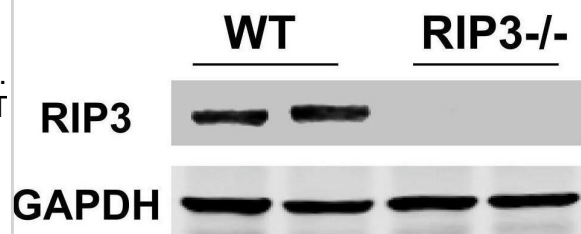


Western Blot: RIPK3/RIP3 Antibody - BSA Free [NBP1-77299]
 - Validation in Human Cell Lines. Loading: 15 ug of lysates per lane. Antibodies: RIPK3/RIP3 , (0.5 ug/mL), 1h incubation at RT in 5% NFDN/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.

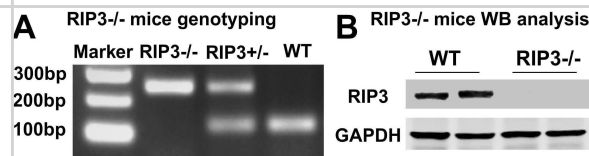


RIP3^{-/-} mice exhibit a decrease in kidney injury after renal I/R. (A) Genotype used to confirm the RIP3^{+/-} and RIP3^{-/-} mice versus the littermate WT mouse. Note that the RIP3^{+/-} heterozygote mouse displayed both alleles (knockout and WT bands). (B) Immunoblot confirming the knockdown of RIP3 in RIP3^{-/-} versus littermate WT mice. (C) Left: H&E staining images showing an increase in kidney injury in WT mice after renal I/R compared to the sham-operated animals. However, in RIP3^{-/-} mice, the tubular injury was reduced when compared to littermate WT mice after renal I/R. Right: Graph showing an increase in kidney injury score in WT mice after renal I/R compared to the sham-operated animals. However, in RIP3^{-/-} mice, the tubular injury was reduced when compared to littermate WT mice after renal I/R. Note that there was no difference in kidney injury between WT and RIP3^{-/-} sham mice. In addition, the cell necrosis, loss of the brush border, cast formation, and tubular dilatation were defined as: 0, none; 1, $\leq 10\%$; 2, 11%–25%; 3, 26%–45%; 4, 46%–75%; 5, >76%. Values are expressed as means \pm SEM; * $p < 0.05$ versus WT group; # $p < 0.05$ versus WT-I/R group, (n = 6/group). (D) Graph showing increases in the levels of serum creatine in WT mice after renal I/R compared to sham-operated animals. However, in RIP3^{-/-} mice, the level of serum creatine was reduced when compared to littermate WT mice after renal I/R. Note that there was no difference in the levels of serum creatine between WT and RIP3^{-/-} sham mice. Values are expressed as means \pm SEM; * $p < 0.05$ versus WT-Sham group, respectively; # $p < 0.05$ versus WT-I/R group (n = 6/group). Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/35741025>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

B RIP3^{-/-} mice WB analysis



RIP3^{-/-} mice exhibit a decrease in kidney injury after renal I/R. (A) Genotype used to confirm the RIP3^{+/-} and RIP3^{-/-} mice versus the littermate WT mouse. Note that the RIP3^{+/-} heterozygote mouse displayed both alleles (knockout and WT bands). (B) Immunoblot confirming the knockdown of RIP3 in RIP3^{-/-} versus littermate WT mice. (C) Left: H&E staining images showing an increase in kidney injury in WT mice after renal I/R compared to the sham-operated animals. However, in RIP3^{-/-} mice, the tubular injury was reduced when compared to littermate WT mice after renal I/R. Right: Graph showing an increase in kidney injury score in WT mice after renal I/R compared to the sham-operated animals. However, in RIP3^{-/-} mice, the tubular injury was reduced when compared to littermate WT mice after renal I/R. Note that there was no difference in kidney injury between WT and RIP3^{-/-} sham mice. In addition, the cell necrosis, loss of the brush border, cast formation, and tubular dilatation were defined as: 0, none; 1, $\leq 10\%$; 2, 11%–25%; 3, 26%–45%; 4, 46%–75%; 5, >76%. Values are expressed as means \pm SEM; * $p < 0.05$ versus WT group; # $p < 0.05$ versus WT-I/R group, (n = 6/group). (D) Graph showing increases in the levels of serum creatine in WT mice after renal I/R compared to sham-operated animals. However, in RIP3^{-/-} mice, the level of serum creatine was reduced when compared to littermate WT mice after renal I/R. Note that there was no difference in the levels of serum creatine between WT and RIP3^{-/-} sham mice. Values are expressed as means \pm SEM; * $p < 0.05$ versus WT-Sham group, respectively; # $p < 0.05$ versus WT-I/R group (n = 6/group). Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/35741025>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Hwang, CH;Lee, M;Kim, JW;Nam, YW;Hwang, G;Ryu, HS;Seo, J;Lee, EW;Ko, HW;Song, J; Opposing regulation of the K63-linked polyubiquitination of RIPK3 by SMURF1 and USP5 in necroptosis *Nature communications* 2025-08-09 [PMID: 40783390]

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] (Western Blot, Human, Mouse)

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] (Western Blot, Human, 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, Human, Mouse)

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] (Western Blot, Human, Mouse)

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] (Western Blot, Human, Mouse)

Lee J, Lee S, Min S, Kang SW. RIP3-Dependent Accumulation of Mitochondrial Superoxide Anions in TNF-?-Induced Necroptosis *Molecules and Cells* 2022-04-30 [PMID: 35289306] (Western Blot, Human, Mouse)

Hänggi K, Li J, Gangadharan A, Liu X et Al. Interleukin-1? release during necrotic-like cell death generates myeloid-driven immunosuppression that restricts anti-tumor immunity *Cancer Cell* 2024-11-22 [PMID: 39577420]

Nam, YW;Shin, JH;Kim, S;Hwang, CH;Lee, CS;Hwang, G;Kim, HR;Roe, JS;Song, J; EGFR inhibits TNF-?-mediated pathway by phosphorylating TNFR1 at tyrosine 360 and 401 *Cell death and differentiation* 2024-05-24 [PMID: 38789573]

Chen XC, Huang LF, Tang JX et Al. Asiatic acid alleviates cisplatin-induced renal fibrosis in tumor-bearing mice by improving the TFEB-mediated autophagy-lysosome pathway *Biomed Pharmacother* 2023-08-17 [PMID: 37413899]

Liu Z, Sun L, Li L et Al. *Pseudomonas aeruginosa* Mediates Host Necroptosis through Rhl-Pqs Quorum Sensing Interaction *Immunohorizons* 2024-09-01 [PMID: 39312394]

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]

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HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations

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