abcam

ab264468

Anti-NLRP3 antibody [EPR23094-1] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR23094-1
Purification technique	Affinity purification Protein A
Specificity	NLRP3 is related with inflammation and is cell-type dependent. This antibody detects no signal or very weak signal in most non-diseased tissue samples and untreated cell lysates. Stimulation may be required to allow detection of the NLRP3 protein in most samples, as described in the literature (PMID: 29070054, 29434227, and 22569257). FURTHER INFORMATION ON SPECIFICITY (Chinese Version) available under the product protocols section. This file includes key technical notes of experience when using this product.
Concentration	1.04 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	Stimulation may be required to allow detection of the NLRP3 protein in most samples. There may be multiple bands of NLRP3 in WB assay due to different isoforms and PTMs.

Species	Mouse
Dilution info	1/1000
Notes	Stimulation may be required to allow detection of the NLRP3 protein in most samples. There may be multiple bands of NLRP3 in WB assay due to different isoforms and PTMs.

Species	Rat
Dilution info	1/1000
Notes	Stimulation may be required to allow detection of the NLRP3 protein in most samples. There may be multiple bands of NLRP3 in WB assay due to different isoforms and PTMs.

Not recommended

Species	Recombinant full length protein
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/30
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Recombinant full length protein
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Human, Rat, Mouse, Recombinant full length protein
Dilution info	-
Notes	-

ICC/IF

Not recommended

Species	Rat, Mouse, Human, Recombinant full length protein
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Species	Mouse
Dilution info	1/30

Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Recombinant full length protein
Dilution info	-
Notes	-

I-ELISA

Tested

Species	Recombinant full length protein
Dilution info	-
Notes	-

Expected

Species	Human, Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information NLRP3

Function	Sensor component of the NLRP3 inflammasome, which mediates inflammasome activation in response to defects in membrane integrity, leading to secretion of inflammatory cytokines IL1B and IL18 and pyroptosis (PubMed:16407889, PubMed:18403674, PubMed:18604214, PubMed:23582325, PubMed:25686105, PubMed:27929086, PubMed:28656979, PubMed:28847925, PubMed:30487600, PubMed:30612879, PubMed:31086327, PubMed:31086329, PubMed:31189953, PubMed:33231615, PubMed:34133077, PubMed:34341353, PubMed:34512673,

PubMed:36442502, PubMed:40450990). In response to pathogens and other damage-associated signals that affect the integrity of membranes, initiates the formation of the inflammasome polymeric complex composed of NLRP3, CASP1 and PYCARD/ASC (PubMed:16407889, PubMed:18403674, PubMed:27432880, PubMed:28847925, PubMed:31189953, PubMed:33231615, PubMed:34133077, PubMed:34341353, PubMed:36142182, PubMed:36442502). Recruitment of pro-caspase-1 (proCASP1) to the NLRP3 inflammasome promotes caspase-1 (CASP1) activation, which subsequently cleaves and activates inflammatory cytokines IL1B and IL18 and gasdermin-D (GSDMD), promoting cytokine secretion and pyroptosis (PubMed:23582325, PubMed:28847925, PubMed:31189953, PubMed:33231615, PubMed:34133077, PubMed:34341353). Activation of NLRP3 inflammasome is also required for HMGB1 secretion; stimulating inflammatory responses (PubMed:22801494). Involved in the homeostatic wound healing response to tissue injury, a multistep cascade that guides neutrophil migration to necrotic sites while avoiding collateral damage of healthy tissues. ATP released from necrotic cells triggers activation of NLRP3 inflammasome through P2RX7 signaling leading to neutrophil adhesion to the vascular endothelium close to the injury site (By similarity). Under resting conditions, ADP-bound NLRP3 is autoinhibited (PubMed:35114687). NLRP3 activation stimuli include extracellular ATP, nigericin, reactive oxygen species, crystals of monosodium urate or cholesterol, amyloid-beta fibers, environmental or industrial particles and nanoparticles, such as asbestos, silica, aluminum salts, cytosolic dsRNA, etc (PubMed:16407889, PubMed:18403674, PubMed:18604214, PubMed:19414800, PubMed:23871209). Almost all stimuli trigger intracellular K(+) efflux (By similarity). These stimuli lead to membrane perturbation and activation of NLRP3 (By similarity). Upon activation, NLRP3 is transported to microtubule organizing center (MTOC), where it is unlocked by NEK7, leading to its relocalization to dispersed trans-Golgi network (dTGN) vesicle membranes and formation of an active inflammasome complex (PubMed:36442502, PubMed:39173637). Associates with dTGN vesicle membranes by binding to phosphatidylinositol 4-phosphate (PtdIns4P) (PubMed:30487600, PubMed:34554188). Shows ATPase activity (PubMed:17483456). Independently of inflammasome activation, regulates the differentiation of T helper 2 (Th2) cells and has a role in Th2 cell-dependent asthma and tumor growth (By similarity). During Th2 differentiation, required for optimal IRF4 binding to IL4 promoter and for IRF4-dependent IL4 transcription (By similarity). Binds to the consensus DNA sequence 5'-GRRGGNRGAG-3' (By similarity). May also participate in the transcription of IL5, IL13, GATA3, CCR3, CCR4 and MAF (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab264468 is the carrier-free version of ab263899.

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 the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	NLRP3 also known as NALP3 or cryopyrin is a protein important for the inflammatory response. It is a component of the inflammasome which senses pathogenic microorganisms and stress. NLRP3 weighs around 118 kDa and is widely expressed in innate immune cells particularly macrophages and dendritic cells. Researchers often use techniques such as 'NLRP3 WB' (Western blot) and 'NLRP3 immunofluorescence' to study NLRP3 expression and localization.
Biological function summary	NLRP3 is central to the body’s defense mechanism by participating in forming the inflammasome complex which activates caspase-1. This activation leads to the cleavage of pro-inflammatory cytokines like IL-1? and IL-18 into their active forms. NLRP3's function is essential for mounting an effective immune response to infections and damage signals. It senses a range of stimuli including crystalline substances and cellular stress triggering an immune response. Its expression is tightly regulated to prevent excessive inflammation.
Pathways	The NLRP3 inflammasome is key in the innate immune response and inflammation pathways. It links cellular stress signals to inflammation through the activation of caspase-1 which is important for the IL-1 signaling pathway. NLRP3 interacts with

	other proteins like ASC (apoptosis-associated speck-like protein containing a CARD) to form the inflammasome complex which is a core part of the inflammatory signaling pathways and links innate and adaptive immunity.
Associated diseases and disorders	NLRP3 has a significant role in inflammatory diseases and is linked to conditions like cryopyrin-associated periodic syndromes (CAPS) and gout. Mutations or dysregulation of NLRP3 lead to hyperactivation resulting in autoimmune and autoinflammatory conditions. NLRP3's interaction with IL-1 contributes to the excessive inflammatory response seen in these disorders. Understanding NLRP3's function in these pathways can inform therapeutic approaches targeting chronic inflammatory diseases.

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

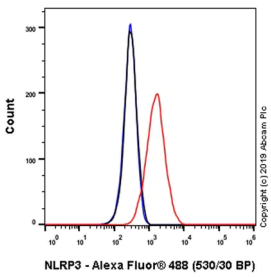
We do not recommend this combination. It is not covered by our product promise.

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.

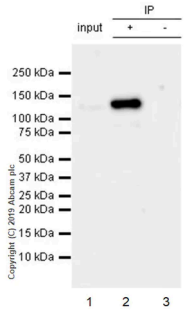
12 product images



Flow Cytometry (Intracellular) - Anti-NLRP3 antibody [EPR23094-1] - BSA and Azide free (ab264468)

NLRP3 Flow Cytometry (Intracellular) staining using rabbit Anti-NLRP3 antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized THP-1 (Human monocytic leukemia monocyte) cells labeling NLRP3 with ab263899 at 1/50 (Red) compared with a Rabbit monoclonal IgG (ab172730) / Black isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor®488, ab150077) at 1/2000 dilution was used as the secondary antibody. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab263899).



Immunoprecipitation - Anti-NLRP3 antibody [EPR23094-1] - BSA and Azide free (ab264468)

NLRP3 was immunoprecipitated from 0.35 mg THP-1 (Human monocytic leukemia monocyte) whole cell lysate 10µg with ab263899 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab263899 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/1000 dilution.
Lane 1: THP-1 whole cell lysate 10µg

Lane 2: ab263899 IP in THP-1 whole cell lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab263899 in THP-1 whole cell lysate

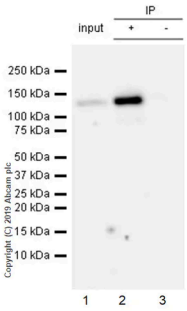
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 30 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab263899).

All lanes:
Immunoprecipitation - Anti-NLRP3 antibody [EPR23094-1] (ab263899)

Predicted band size: 118 kDa



Immunoprecipitation - Anti-NLRP3 antibody [EPR23094-1] - BSA and Azide free (ab264468)

NLRP3 was immunoprecipitated from 0.35 mg RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate 10µg with ab263899 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab263899 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/1000 dilution.
Lane 1: RAW 264.7 whole cell lysate 10µg

Lane 2: ab263899 IP in RAW 264.7 whole cell lysate

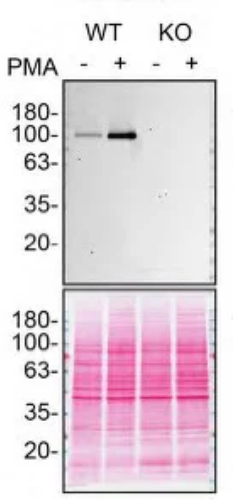
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab263899 in RAW 264.7 whole cell lysate

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

Exposure time: 10 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab263899).
All lanes:
Immunoprecipitation - Anti-NLRP3 antibody [EPR23094-1] (ab263899)

Predicted band size: 118 kDa



Western blot - Anti-NLRP3 antibody [EPR23094-1] - BSA and Azide free (ab264468)

NLRP3 Western blot staining using rabbit Anti-NLRP3 antibody

This data was developed using ab263899, the same antibody clone in a different buffer formulation.

ab263899 was shown to react with NLRP3 in wild-type THP-1 cells in Western blot with loss of signal observed in a NLRP3 knockout cell line. Wild-type THP-1 and NLRP3 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab263899 overnight at 4 °C at a 1/1000 dilution. Blots were incubated with secondary antibodies at 0.2 µg/mL before imaging.

These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.

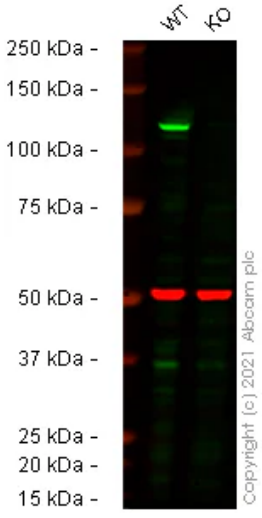
All lanes:
Western blot - Anti-NLRP3 antibody [EPR23094-1] (ab263899) at 1/1000 dilution

Lane 1:
Wild-type THP-1 lysate at 20 µg

Lane 2:
Wild-type THP-1 lysate treated with 200 ng/ml PMA, 48 hr at 20 µg

Lane 3:
NLRP3 knock-out THP-1 lysate at 20 µg

Lane 4:
NLRP3 knock-out THP-1 lysate treated with 200 ng/ml PMA, 48 hr at 20 µg



Western blot - Anti-NLRP3 antibody [EPR23094-1] - BSA and Azide free (ab264468)

NLRP3 Western blot staining using rabbit Anti-NLRP3 antibody

False colour image of Western blot: Anti-NLRP3 antibody [EPR23094-1] staining at 1/500 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab263899 was shown to bind specifically to NLRP3. A band was observed at 118 kDa in wild-type THP-1 cell lysates with no signal observed at this size in NLRP3 knockout cell line ab280063 (knockout cell lysate ab280122). To generate this image, wild-type and NLRP3 knockout THP-1 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were

Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (ab216772) at 1/20000 dilution.
All lanes:
Western blot - Anti-NLRP3 antibody [EPR23094-1] (ab263899) at 1/500 dilution

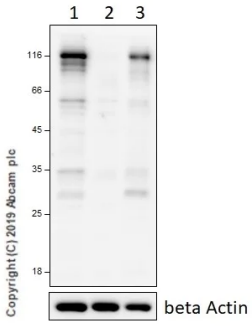
Lane 1:
Wild-type THP-1 cell lysate at 20 µg

Lane 2:
Western blot - Human NLRP3 knockout THP-1 cell lysate (ab280122) at 20 µg

Performed under reducing conditions.

Predicted band size: 118 kDa

Observed band size: 118 kDa



This image is courtesy of Benedikt Saller, from Prof. Dr. Olaf Groß' lab at the University of Freiburg.

Western blot - Anti-NLRP3 antibody [EPR23094-1] - BSA and Azide free (ab264468)

Blocking step: 2% SMP in PBS/T for 60 minutes at RT.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab263899).

All lanes:
Western blot - Anti-NLRP3 antibody [EPR23094-1] (ab263899) at 1/1000 dilution

Lane 1:
Wild-type mouse BMDCs treated with 50 ng/ml LPS at 25000 Cells

Lane 2:
NLRP3 knockout mouse BMDCs treated with 50 ng/ml LPS at 25000 Cells

Lane 3:
Wild-type human THP1 cells treated with 200 ng/ml LPS at 25000 Cells

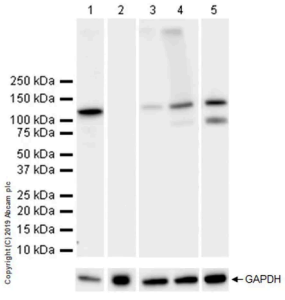
Secondary

All lanes:
Goat anti-Rabbit HRP at 1/5000 dilution

Developed using the ECL technique.

Predicted band size: 118 kDa

Exposure time: 10s



Western blot - Anti-NLRP3 antibody [EPR23094-1] - BSA and Azide free (ab264468)

The molecular weight/expression profile observed is consistent with what has been described in the literature (PMID: 26939933, 30315268).
Note: The band around 75kDa is the short form of NLRP3 (NLRP3s).

Low expression cell line: Jurkat (PMID: 19767079).

Exposure times: Lane 1: 48 secs; Lane 2: 3 min; Lanes 3-4: 1 sec; Lane 5: 6 secs.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab263899).
All lanes:
Western blot - Anti-NLRP3 antibody [EPR23094-1] (ab263899) at 1/1000 dilution

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

Lane 2:
Jurkat (human t cell leukemia t lymphocyte) whole cell lysate at 20 µg

Lane 3:
Untreated RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 10 µg

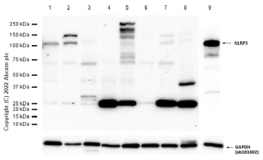
Lane 4:
RAW 264.7 treated with 10 µg/ml LPS for 8 hours, whole cell lysate at 10 µg

Lane 5:
J774A.1 (mouse reticulum cell sarcoma monocyte macrophage) whole cell lysate at 20 µg

Secondary

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

Predicted band size: 118 kDa



Western blot - Anti-NLRP3 antibody [EPR23094-1] - BSA and Azide free (ab264468)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab263899). Blocking and Diluting buffer: 5% NFDM/TBST Lane 9 Exposure Time: 20 seconds NLRP3 is expressed at a much low level in normal tissues (PMID: 33538177, PMID: 25524927, PMID: 20688930, PMID: 35676979, PMID: 31923221, PMID: 24166187).
All lanes:
Western blot - Anti-NLRP3 antibody [EPR23094-1] (ab263899) at 1/1000 dilution

Lane 1:
Mouse thymus tissue lysate at 20 µg

Lane 2:
Mouse lung tissue lysate at 20 µg

Lane 3:
Mouse heart tissue lysate at 20 µg

Lane 4:
Mouse kidney tissue lysate at 20 µg

Lane 5:
Mouse brain tissue lysate at 20 µg

Lane 6:
Mouse placenta tissue lysate at 20 µg

Lane 7:
Mouse ovary tissue lysate at 20 µg

Lane 8:
Mouse liver tissue lysate at 20 µg

Lane 9:
Raw 264.7(Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

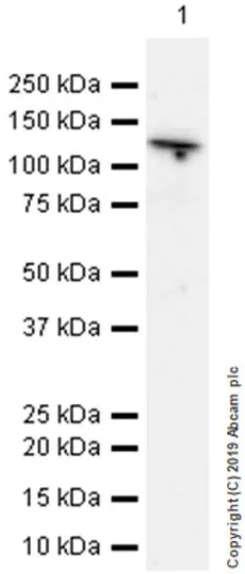
Secondary

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

Predicted band size: 118 kDa

Observed band size: 118 kDa

Exposure time: 180s



Western blot - Anti-NLRP3 antibody [EPR23094-1] - BSA and Azide free (ab264468)

NLRP3 Western blot staining of Rat spleen lysate using rabbit Anti-NLRP3 antibody

The molecular weight/expression profile observed is consistent with what has been described in the literature (PMID: 26939933, 30315268).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab263899).

All lanes:
Western blot - Anti-NLRP3 antibody [EPR23094-1] (ab263899) at 1/1000 dilution

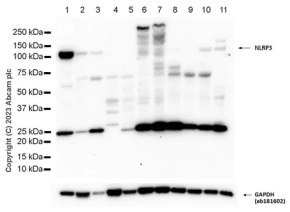
All lanes:
Rat spleen lysate at 10 µg

Secondary

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

Predicted band size: 118 kDa

Exposure time: 3min



Western blot - Anti-NLRP3 antibody [EPR23094-1] - BSA and Azide free (ab264468)

NLRP3 Western blot staining using rabbit Anti-NLRP3 antibody

Blocking and dilution buffer: 5% NFDM /TBST. NLRP3 is expressed at a much low level in normal tissues (PMID: 33538177, PMID: 25524927, PMID: 20688930, PMID: 35676979, PMID: 31923221, PMID: 24166187).
All lanes:
Western blot - Anti-NLRP3 antibody [EPR23094-1] (ab263899) at 1/1000 dilution

Lane 1:
Rat spleen tissue lysate at 20 µg

Lane 2:

Rat thymus tissue lysate at 20 µg

Lane 3:
Rat lung tissue lysate at 20 µg

Lane 4:
Rat heart tissue lysate at 20 µg

Lane 5:
Rat kidney tissue lysate at 20 µg

Lane 6:
Rat brain tissue lysate at 20 µg

Lane 7:
Rat hippocampus tissue lysate at 20 µg

Lane 8:
Rat spinal cord tissue lysate at 20 µg

Lane 9:
Rat placenta tissue lysate at 20 µg

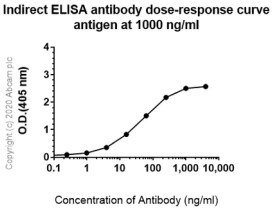
Lane 10:
Rat ovary tissue lysate at 20 µg

Lane 11:
Rat liver tissue lysate at 20 µg

Secondary

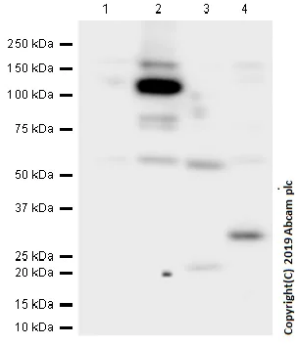
All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Exposure time: 180s



Indirect ELISA - Anti-NLRP3 antibody [EPR23094-1] - BSA and Azide free (ab264468)

This data was developed using ab263899, the same antibody clone in a different buffer formulation.ELISA analysis of NLRP3 recombinant protein at 1000 ng/mL with ab263899. An Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) at 1/2500 dilution was used as the secondary antibody.



Western blot - Anti-NLRP3 antibody [EPR23094-1] - BSA and Azide free (ab264468)

All lanes:
Western blot - Anti-NLRP3 antibody [EPR23094-1] (ab263899) at 1/1000 dilution

Lane 1:
C6 (Rat glial tumor glial cell) whole cell lysate at 10 µg

Lane 2:
RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 10 µg

Lane 3:

PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate at 10 µg

Lane 4:
NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/10000 dilution

Predicted band size: 118 kDa

Observed band size: 118 kDa

Exposure time: 15s

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