abcam

ab314905

Anti-NLRP3 antibody [RM1021] - 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	Multiclonal
Immunogens	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	RM1021
Purification technique	Affinity purification Protein A
Specificity	Expression levels of NLRP3 may vary with sample type and stimulation may be required to allow detection. It is recommended to test varying time and concentration gradients to optimize induction.
Concentration	0.57 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human

Dilution info	-
Notes	-

Species	Mouse
Dilution info	-
Notes	After extending the induction period to 16 hours, prolonged LPS stimulation may trigger cellular feedback regulation (PMID: 26412089), resulting in less pronounced induction effects. It is recommended to test varying time and concentration gradients to optimize induction.

Species	Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human, Mouse
Dilution info	-
Notes	-

Expected

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

Flow Cyt (Intra)

Tested

Species	Human, Mouse
Dilution info	-
Notes	-

Expected

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

IP

Tested

Species	Human, Mouse
Dilution info	-
Notes	-

Expected

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

IHC-P

Not recommended

Species	Human, Mouse, Rat
Dilution info	-
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

Notes

ab314905 is the carrier-free version of ab283819.

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.

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.

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-1β 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

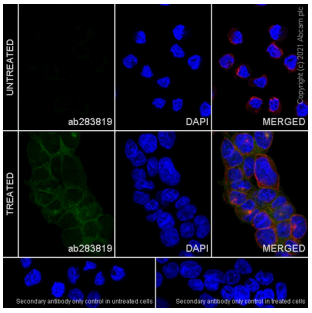
We do not recommend this combination. It is not covered by our product promise.

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.

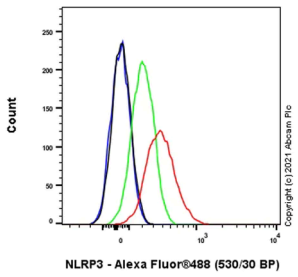
9 product images



Immunocytochemistry/ Immunofluorescence - Anti-NLRP3 antibody [RM1021] - BSA and Azide free (ab314905)

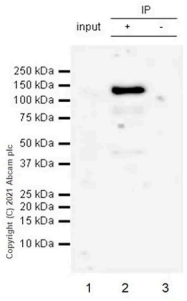
This data was developed using ab283819, the same antibody clone in a different buffer formulation. Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized THP-1 (human monocytic leukemia monocyte) cells labelling NLRP3 with ab283819 at 1/50 (11.4 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2ug/ml dilution (Green). Confocal image showing increased mainly cytoplasmic staining in THP-1 cells treated with 12-O-Tetradecanoylphorbol-13-acetate (80 nM) for 16 h then replaced it with lipopolysaccharide (1 ug/ml) for 8 h. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2ug/ml dilution.



Flow Cytometry (Intracellular) - Anti-NLRP3 antibody [RM1021] - BSA and Azide free (ab314905)

This data was developed using ab283819, the same antibody clone in a different buffer formulation. Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized THP-1 (Human monocytic leukemia monocyte) treated with 50nM PMA and 5ug/ml LPS for 24h(Red) / Untreated control (Green) cells labelling NLRP3 with ab283819 at 1/50 dilution (1ug) (Red) and Green (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-NLRP3 antibody [RM1021] - BSA and Azide free (ab314905)

This data was developed using ab283819, the same antibody clone in a different buffer formulation. NLRP3 was immunoprecipitated from 0.35 mg THP-1 (Human monocytic leukemia monocyte) whole cell lysate 10ug with ab283819 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab283819 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 10 µg Lane 2: ab283819 IP in THP-1 (Human monocytic leukemia monocyte) whole cell lysate Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab283819 in THP-1 whole cell lysate Blocking and dilution buffer and concentration: 5% NFDM/TBST. Lower bands should be cleavage fragment of NLRP3. (PMID: 26119741)
All lanes:
Immunoprecipitation - Anti-NLRP3 antibody [RM1021] (ab283819) at 1/30 dilution

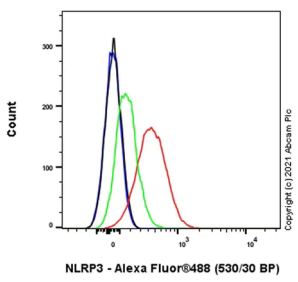
Lane 1:
THP-1 (Human monocytic leukemia monocyte) whole cell lysate

Lane 2:
ab283819 IP in THP-1 (Human monocytic leukemia monocyte) whole cell lysate

Secondary

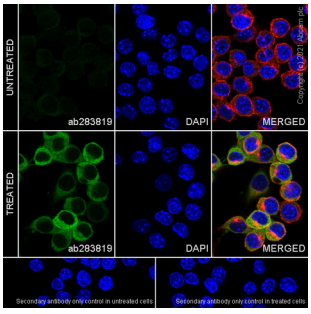
All lanes:
Immunoprecipitation - Anti-NLRP3 antibody [RM1021] (ab283819) at 1/5000 dilution

Exposure time: 84s



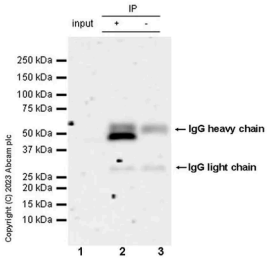
Flow Cytometry (Intracellular) - Anti-NLRP3 antibody [RM1021] - BSA and Azide free (ab314905)

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Immunocytochemistry/ Immunofluorescence - Anti-NLRP3 antibody [RM1021] - BSA and Azide free (ab314905)

This data was developed using ab283819, the same antibody clone in a different buffer formulation. Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) cells labelling NLRP3 with ab283819 at 1/50 (11.4 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2ug/ml dilution (Green). Confocal image showing increased cytoplasmic staining in RAW 264.7 cells treated with lipopolysaccharide (10 ug/ml) for 8 h. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Red). The Nuclear counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2ug/ml dilution.



Immunoprecipitation - Anti-NLRP3 antibody [RM1021] - BSA and Azide free (ab314905)

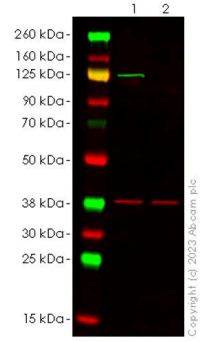
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All lanes:
Immunoprecipitation - Anti-NLRP3 antibody [RM1021] (ab283819) at 1/30 dilution

All lanes:
RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate

Secondary

All lanes:
Immunoprecipitation - Anti-NLRP3 antibody [RM1021] (ab283819) at 1/5000 dilution

Exposure time: 84s



Western blot - Anti-NLRP3 antibody [RM1021] - BSA and Azide free (ab314905)

This data was developed usingab283819, the same antibody clone in a different buffer formulation. Blocking and diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of TBS Lysates at 20 µg per lane. The samples were run on a Bis-Tris gel under reducing conditions. Western blot: Anti-NLRP3 antibody (ab283819) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody 6C5 (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab283819 was shown to bind specifically to NLRP3. Target of interest was observed at 118 kDa in untreated wild-type THP-1 cell lysates (lane 1) with no signal observed at this size in NLRP3 knockout cell line ab280063 (knockout cell lysate ab280122) (lane 2). To generate this image, samples were first run on an SDS-PAGE gel then transferred onto an immobilon-FL PVDF membrane. Membranes were blocked in a fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed in TBS-T, incubated with secondary antibodies Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution for 1 h at room temperature, washed again then imaged.
All lanes:
Western blot - Anti-NLRP3 antibody [RM1021] (ab283819) at 1/1000 dilution

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

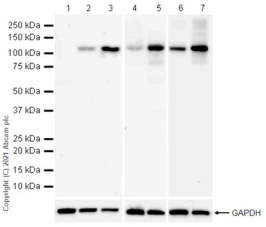
Lane 2:
NLRP3 knockout THP-1 whole cell lysate at 20 µg

Secondary

Lanes 1 - 2:
Goat Anti-Rabbit IgG H&L (800CW) at 1/20000 dilution

Lanes 1 - 2:
Goat anti-Mouse IgG H&L (680RD) at 1/20000 dilution

Observed band size: 118 kDa, 37 kDa



Western blot - Anti-NLRP3 antibody [RM1021] - BSA and Azide free (ab314905)

This data was developed usingab283819, the same antibody clone in a different buffer formulation. Blocking and diluting buffer and concentration: 5% NFDM/TBST Negative control: Jurkat (PMID:19767079). Exposure time: Lane1-3: 3min; Lane4-5: 37 seconds; Lane6-7: 10 seconds
All lanes:
Western blot - Anti-NLRP3 antibody [RM1021] (ab283819) at 1/1000 dilution

Lane 1:
Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate at 20 µg

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

Lane 3:
THP-1 treated with 80nM TPA overnight, then 1µg/ml lipopolysaccharide (LPS) for 8h whole cell lysate at 20 µg

Lane 4:
RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Lane 5:
RAW 264.7 treated with 1µg/ml lipopolysaccharide (LPS) for 6h whole cell lysate at 20 µg

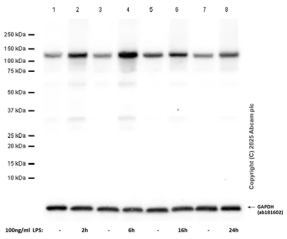
Lane 6:
NR8383 (Rat lu macrophage (alveolar)) whole cell lysate at 20 µg

Lane 7:
NR8383 treated with 1µg/ml lipopolysaccharide (LPS) for 4h, then add 1µg/ml BFA for another 3h whole cell lysate at 20 µg

Secondary

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

Observed band size: 118 kDa



Western blot - Anti-NLRP3 antibody [RM1021] - BSA and Azide free (ab314905)

NLRP3 Western blot staining using rabbit Anti-NLRP3 antibody

This data was developed using ab283819, the same antibody clone in a different buffer formulation.
In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/1000000 dilution.

After extending the induction period to 16 hours, prolonged LPS stimulation may trigger cellular feedback regulation (PMID: 26412089), resulting in less pronounced induction effects. It is recommended to test varying time and concentration gradients to optimize induction.

All lanes:
Western blot - Anti-NLRP3 antibody [RM1021] (ab283819) at 1/1000 dilution

Lanes 1, 3, 5 and 7:
RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Lane 2:
RAW 264.7 treated with 100ng/ml lipopolysaccharide (LPS) for 2h whole cell lysate at 20 µg

Lane 4:
RAW 264.7 treated with 100ng/ml lipopolysaccharide (LPS) for 6h whole cell lysate at 20 µg

Lane 6:
RAW 264.7 treated with 100ng/ml lipopolysaccharide (LPS) for 16h whole cell lysate at 20 µg

Lane 8:
RAW 264.7 treated with 100ng/ml lipopolysaccharide (LPS) for 24h whole cell lysate at 20 µg

Secondary

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

Observed band size: 118 kDa

Exposure time: 3s

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