

Datasheet for 600-401-R14**NLRP3 Antibody****Overview**

Description:	Anti-NLRP3 (RABBIT) Antibody - 600-401-R14
Item No.:	600-401-R14
Size:	100 µg
Applications:	FC, IF, IHC, WB
Reactivity:	Human, Mouse, Rat
Host Species:	Rabbit

Product Details

Background: NLRP3(NLR FAMILY, PYRIN DOMAIN-CONTAINING 3),also known as CIAS1, CRYOPYRIN, NALP3 or PYPAF1, is a protein that in humans is encoded by the NLRP3 (NOD-like receptor family, pyrin domain containing 3) gene. The NLRP3 gene encodes a pyrin-like protein expressed predominantly in peripheral blood leukocytes. And the NLRP3 gene is mapped on 1q44. NLRP3 interacts with apoptosis-associated speck-like protein containing a CARD (ASC). The encoded protein may play a role in the regulation of inflammation and apoptosis. Mutation of the NALP3 nucleotide-binding domain reduced ATP binding, CASP1 activation, IL1B production, cell death, macromolecular complex formation, self-association, and association with ASC. Consistent with an essential role for Nlrp3 inflammasomes in antifungal immunity, Gross et al. showed that Nlrp3-deficient mice are hypersusceptible to *C. albicans* infection. Activation of the NLRP3 inflammasome in response to virus or to RNA was dependent upon lysosomal maturation and reactive oxygen species production in human cells. The NLRP3 inflammasome senses obesity-associated danger signals and contributes to obesity-induced inflammation and insulin resistance. This antibody is suitable for researchers interested in cancer research.

Synonyms:	Angiotensin/vasopressin receptor AII/AVP-like,Caterpillar protein 1.1,
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	NLRP3
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Reactivity:	Human, Mouse, Rat
Immunogen Type:	Conjugated Peptide
Immunogen:	NLRP3 affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to a sequence at the N-terminal of human NLRP3.
Purity/Specificity:	Anti-NLRP3 antibody is directed against human NLRP3 protein. The product was affinity purified from monospecific antiserum by immunoaffinity purification. A BLAST analysis was used to suggest reactivity with this protein from human, mouse, and rat based on homology for the immunogen sequence. Cross reactivity with NLRP3 from other sources has not been determined.
Relevant Links:	• UniProtKB - Q96P20 • GeneID - 114548 • NCBI - NP_001073289.1

Application Details

Tested Applications:	FC, IF, IHC, WB
Application Note:	Anti-NLRP3 is tested for Flow Cytometry, Immunofluorescence, Immunocytochemistry, Immunohistochemistry -P and Western Blotting. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately ~118.2 kDa corresponding to the appropriate cell lysate or extract.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
FC:	1-3µg/1x10 ⁶ cells
IF:	2µg/ml
IHC:	1:100-1:500
WB:	0.5 µg/mL

Formulation

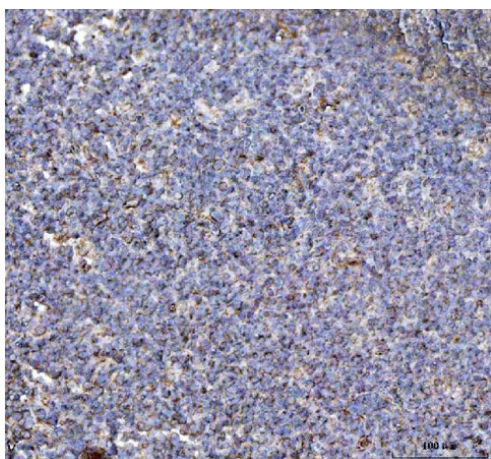
Physical State:	Lyophilized
Concentration:	0.5 mg/mL by UV absorbance at 280 nm
Buffer:	0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg Na ₃
Preservative:	0.05mg Thimerosal
Stabilizer:	5mg BSA

Reconstitution Volume:	200µL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

Shipping & Handling

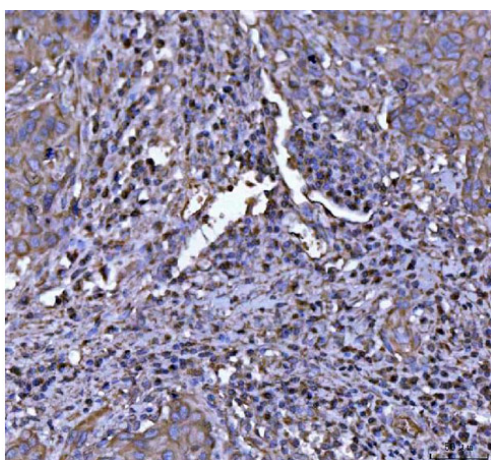
Shipping Condition:	Ambient
Storage Condition:	Store vial at 4° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



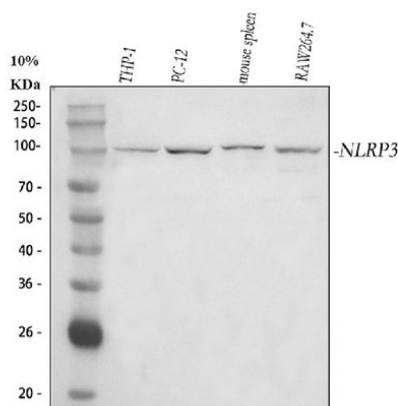
Immunohistochemistry

Immunohistochemistry analysis of NLRP3 using anti-NLRP3 antibody. NLRP3 was detected in a paraffin-embedded section of human tonsil tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-NLRP3 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.



Immunohistochemistry

Immunohistochemistry analysis of NLRP3 using anti-NLRP3 antibody. NLRP3 was detected in a paraffin-embedded section of human squamous cell cervical carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-NLRP3 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.

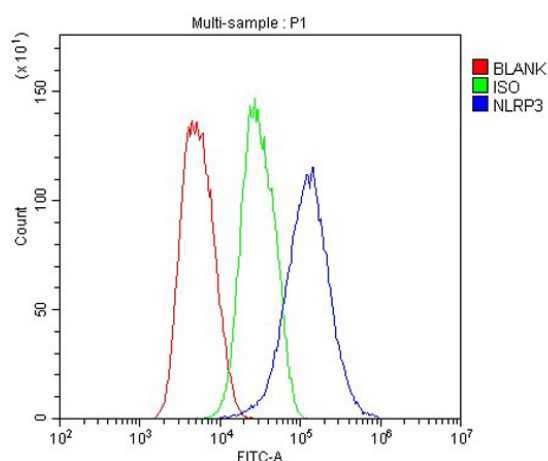


Western Blot

Western blot analysis of NLRP3 using anti-NLRP3 antibody. Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30µg of sample under reducing conditions.

Lane 1: human THP-1 whole cell lysates, Lane 2: rat PC-12 whole cell lysates, Lane 3: mouse spleen whole cell lysates, Lane 4: mouse RAW264.7 whole cell lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with affinity purified rabbit anti-NLRP3 antigen polyclonal antibody at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit with Tanon 5200 system. A specific band was detected for NLRP3 at approximately 110 kDa. The expected band size for NLRP3 is at 118 kDa.



Flow Cytometry

Flow Cytometry analysis of THP-1 cells using anti-NLRP3 antibody.

Overlay histogram showing THP-1 cells stained with (NLRP3, Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-NLRP3 Antibody (p/n 600-401-R14, 1 µg/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 µg/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control rabbit IgG antibody (1 µg/1x10⁶, Green line) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

References

- Long FQ et al. MiR-223-3p inhibits rTp17-induced inflammasome activation and pyroptosis by targeting NLRP3. *J Cell Mol Med.* (2020)

Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.