BOSTER
antibody and ELISA experts

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566

Phone: 888-466-3604 Fax: 925-215-2184 Email:support@bosterbio.com Web: www.bosterbio.com

Anti-CIAS1/NALP3/NLRP3 Antibody

Catalog Number:PA1665

About NLRP3

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, pryin 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.

Overview

Product Name	Anti-CIAS1/NALP3/NLRP3 Antibody
Reactive Species	Human, Mouse, Rat
Description	Rabbit IgG polyclonal antibody for NACHT, LRR and PYD domains-containing protein 3(NLRP3) detection. Tested with WB, IHC-P, ICC/IF, FCM in Human;Mouse;Rat.
Application	Flow Cytometry, IF, IHC-P, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Storage Instructions	At -20°C for one year. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for a longer time.Avoid repeated freezing and thawing.
Host	Rabbit
Uniprot ID	Q96P20

Technical Details

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Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human CIAS1(12-31aa RYLEDLEDVDLKKFKMHLED), different from the related rat and mouse sequences by one amino acid.
Predicted Reactive Species	Hamster
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross reactivity with other proteins
Isotype	N/A
Form	Lyophilized
Concentration	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Immunohistochemistry(Paraffin-embedded Section), 0.5-1µg/ml, Human, Rat, Mouse, By Heat Western blot, 0.1-0.5µg/ml, Human Immunocytochemistry/ Immunofluorescence, 2µg/ml, Human, Rat Flow Cytometry, 1-3µg/1x10^6 cells, Human For protocols please visit https://www.bosterbio.com/protocol-and-troubleshooting/

Anti-CIAS1/NALP3/NLRP3 Antibody (PA1665) Images

Figure 1. Western blot analysis of -CIAS1/NALP3 using anti--CIAS1/NALP3 antibody (PA1665).
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 50ug of sample under reducing conditions.
Lane 1: HEP-2 Cell Lysate
Lane 2: A549 Cell Lysate
Lane 3: U87 Cell Lysate
Lane 4: CEM Cell Lysate
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti--CIAS1/NALP3 antigen affinity purified polyclonal antibody (Catalog # PA1665) at 0.5 Î¼g/mL overnight at 4Â°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary

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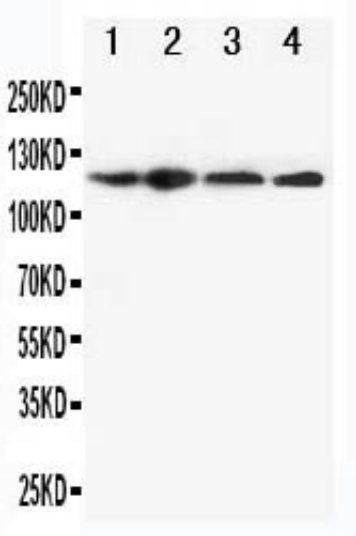

antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for -CIAS1/NALP3 at approximately 118KD. The expected band size for -CIAS1/NALP3 is at 118KD.

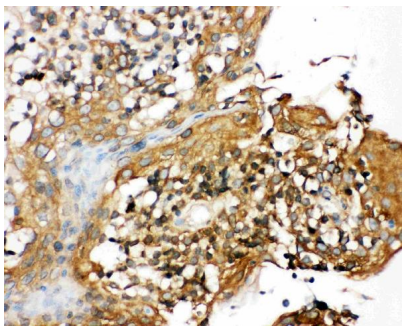

Figure 2. IHC analysis of CIAS1/NALP3 using anti-CIAS1/NALP3 antibody (PA1665).
CIAS1/NALP3 was detected in paraffin-embedded section of human tonsil tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1Î¼g/ml rabbit anti- CIAS1/NALP3 Antibody (PA1665) overnight at 4Â°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37Â°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

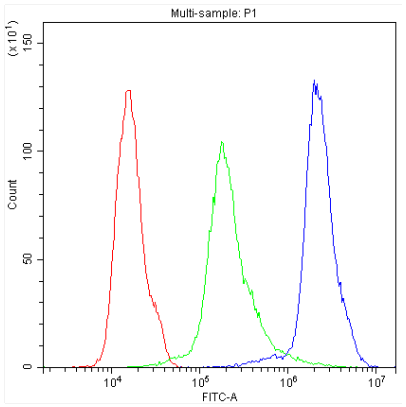

Figure 3. Flow Cytometry analysis of THP-1 cells using anti-CIAS1/NALP3 antibody (PA1665).
Overlay histogram showing THP-1 cells stained with PA1665 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-CIAS1/NALP3 Antibody (PA1665,1Î¼g/1x10^6 cells) for 30 min at 20Â°C. DyLightÂ®488 conjugated goat anti-rabbit IgG (BA1127, 5-10Î¼g/1x10^6 cells) was used as secondary antibody for 30 minutes at 20Â°C. Isotype control antibody (Green line) was rabbit IgG (1Î¼g/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Figure 4. Flow Cytometry analysis of U937 cells using anti-CIAS1/NALP3 antibody (PA1665).
Overlay histogram showing U937 cells stained with PA1665 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-CIAS1/NALP3 Antibody (PA1665,1Î¼g/1x10^6 cells) for 30 min at 20Â°C.

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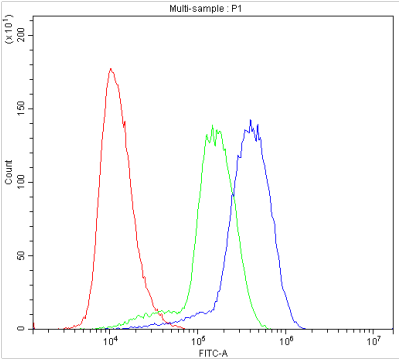


DyLightÂ®488 conjugated goat anti-rabbit IgG (BA1127, 5-10Î¼g/1x10^6 cells) was used as secondary antibody for 30 minutes at 20Â°C. Isotype control antibody (Green line) was rabbit IgG (1Î¼g/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

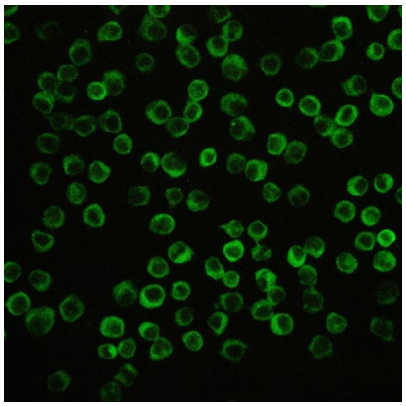


Figure 5. IF analysis of CIAS1/NALP3 using anti- CIAS1/NALP3 antibody (PA1665).
CIAS1/NALP3 was detected in immunocytochemical section of THP-1 cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2Î¼g/mL rabbit anti- CIAS1/NALP3 Antibody (PA1665) overnight at 4Â°C. DyLightÂ®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37Â°C. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

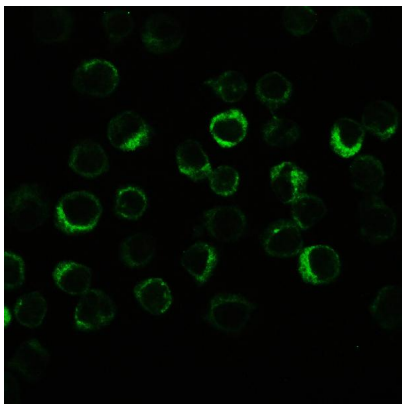
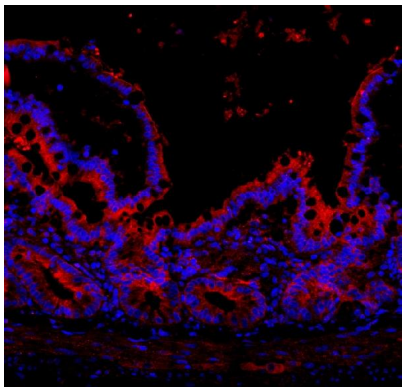


Figure 6. IF analysis of CIAS1/NALP3 using anti- CIAS1/NALP3 antibody (PA1665).
CIAS1/NALP3 was detected in immunocytochemical section of THP-1 cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2Î¼g/mL rabbit anti- CIAS1/NALP3 Antibody (PA1665) overnight at 4Â°C. DyLightÂ®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37Â°C. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

Figure 9. IF analysis of CIAS1/NALP3 using anti- CIAS1/NALP3 antibody (PA1665)
CIAS1/NALP3 was detected in paraffin-embedded section of rat colon tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1Î¼g/mL rabbit anti- CIAS1/NALP3 Antibody (PA1665) overnight at 4Â°C. Cy3 Conjugated Goat Anti-Rabbit IgG (BA1032) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37Â°C. The section was counterstained



with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

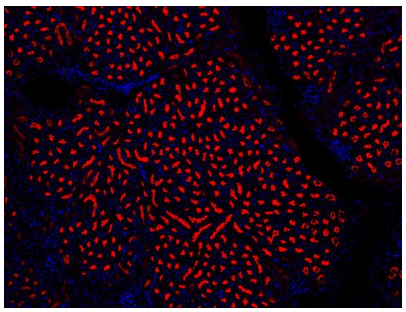


Figure 7. IF analysis of CIAS1/NALP3 using anti- CIAS1/NALP3 antibody (PA1665)
CIAS1/NALP3 was detected in paraffin-embedded section of rat kidney tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1Î¼g/mL rabbit anti- CIAS1/NALP3 Antibody (PA1665) overnight at 4Â°C. Cy3 Conjugated Goat Anti-Rabbit IgG (BA1032) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37Â°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

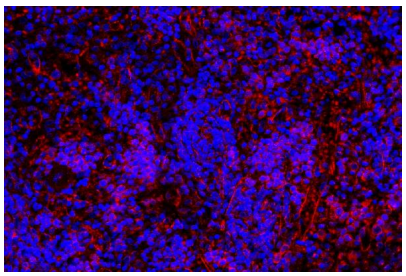


Figure 8. IF analysis of CIAS1/NALP3 using anti- CIAS1/NALP3 antibody (PA1665)
CIAS1/NALP3 was detected in paraffin-embedded section of rat spleen tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1Î¼g/mL rabbit anti- CIAS1/NALP3 Antibody (PA1665) overnight at 4Â°C. Cy3 Conjugated Goat Anti-Rabbit IgG (BA1032) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37Â°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

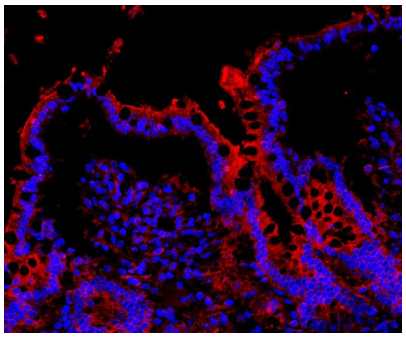


Figure 10. IF analysis of CIAS1/NALP3 using anti- CIAS1/NALP3 antibody (PA1665)
CIAS1/NALP3 was detected in paraffin-embedded section of rat colon tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1Î¼g/mL rabbit anti- CIAS1/NALP3 Antibody (PA1665) overnight at 4Â°C. Cy3 Conjugated Goat Anti-Rabbit IgG (BA1032) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37Â°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and

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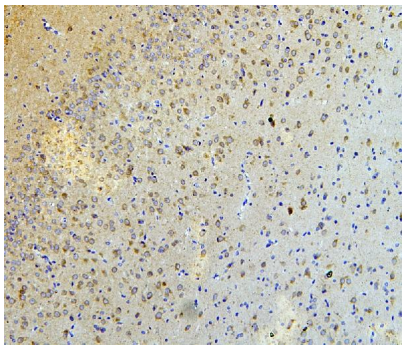

Figure 11. IHC analysis of NLRP3 using anti- NLRP3 antibody (PA1665).
NLRP3 was detected in paraffin-embedded section of rat brain tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1Î¼g/ml rabbit anti- NLRP3 Antibody (PA1665) overnight at 4Â°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37Â°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.