BOSTER BIOLOGICAL TECHNOLOGY, LTD.

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Certificate of Analysis

Note: this is a sample COA. To get the COA for your lot #, please contact us at support@bosterbio.com

To whom it may concern,

This letter attests that Anti-CIAS1/NALP3/NLRP3 Antibody , catalog # PA1665, is manufactured by Boster Biological Technology., Ltd, through test and assay, we got the following data:

1. 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.

2. Application Data

Applications Details

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

3. Cross Reaction

No cross reactivity with other proteins

4. Image

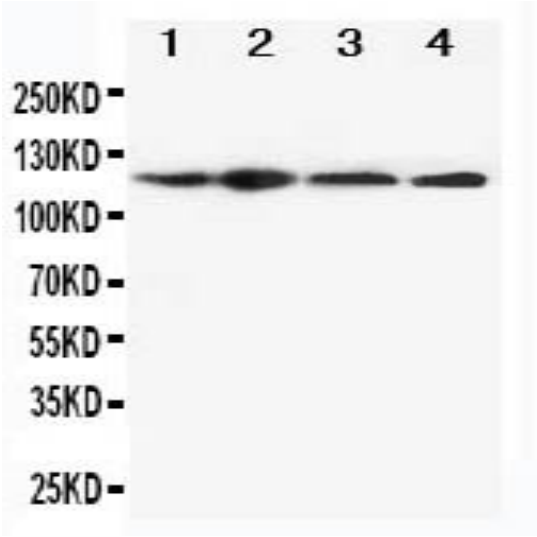

Figure 1. Western blot analysis of -CIAS1/NALP3 using anti- -CIAS1/NALP3 antibody (PA1665). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: HEP-2 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 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.

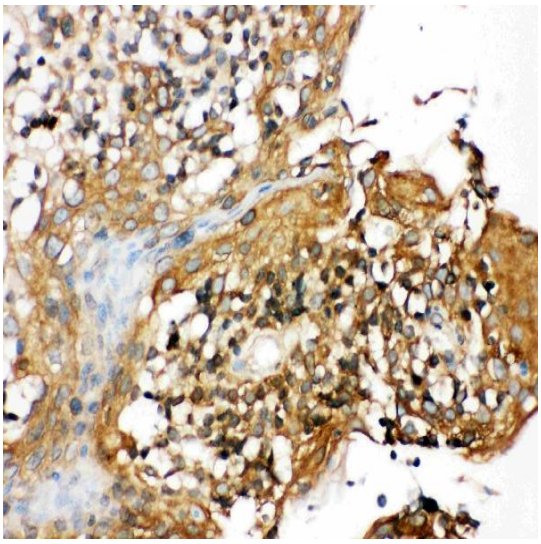

Figure 2. IHC analysis of CIAS1/NALP3 using anti- CIAS1/NALP3 antibody (PA1665). CIAS1/NALP3 was detected in paraffin-embedded section of human tonsil tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked 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.

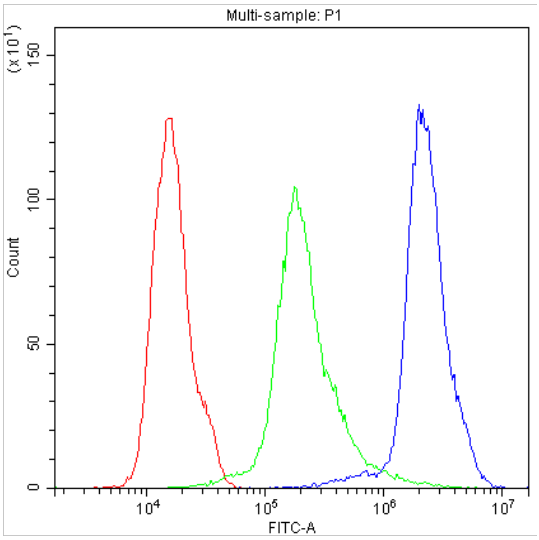

Figure 3. Flow Cytometry analysis of THP-1 cells using anti-CIAS1/NALP3 antibody (PA1665). Overlay histogram showing THP-1 cells stained with PA1665 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-CIAS1/NALP3 Antibody (PA1665,1Î¼g/1x10^6 cells) for 30 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.

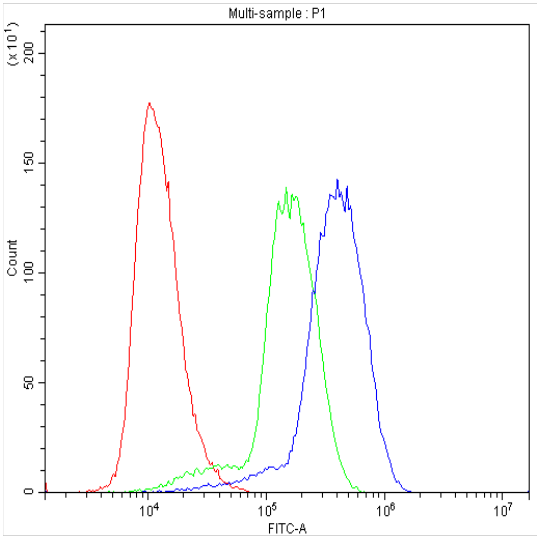

Figure 4. Flow Cytometry analysis of U937 cells using anti-CIAS1/NALP3 antibody (PA1665). Overlay histogram showing U937 cells stained with PA1665 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-CIAS1/NALP3 Antibody (PA1665,1Î¼g/1x10^6 cells) for 30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

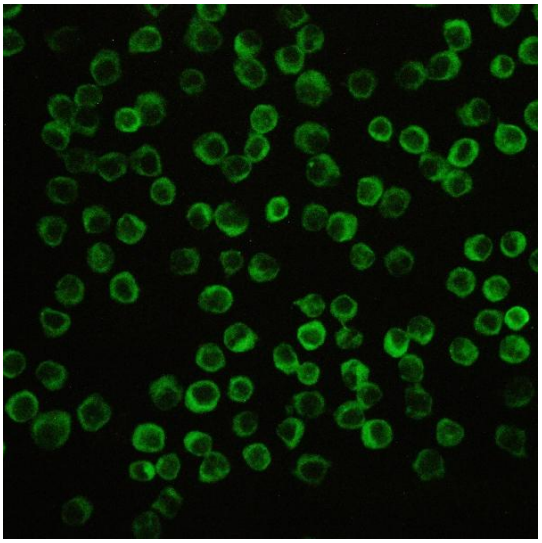

Figure 5. IF analysis of CIAS1/NALP3 using anti- CIAS1/NALP3 antibody (PA1665). CIAS1/NALP3 was detected in immunocytochemical section of THP-1 cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And 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.

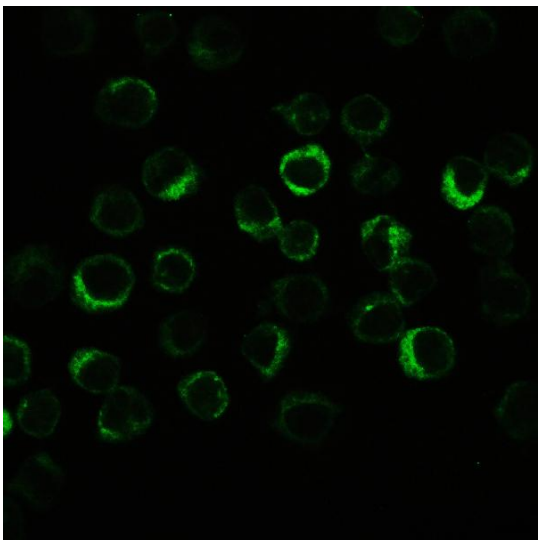

Figure 6. IF analysis of CIAS1/NALP3 using anti- CIAS1/NALP3 antibody (PA1665). CIAS1/NALP3 was detected in immunocytochemical section of THP-1 cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And 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.

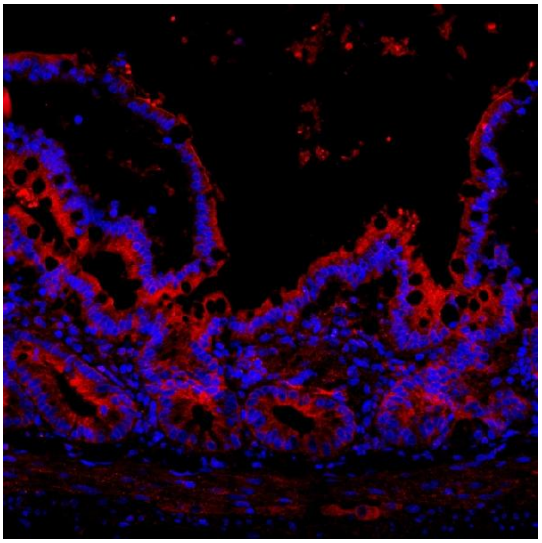

Figure 9. IF analysis of CIAS1/NALP3 using anti- CIAS1/NALP3 antibody (PA1665)
CIAS1/NALP3 was detected in paraffin-embedded section of rat colon tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 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.

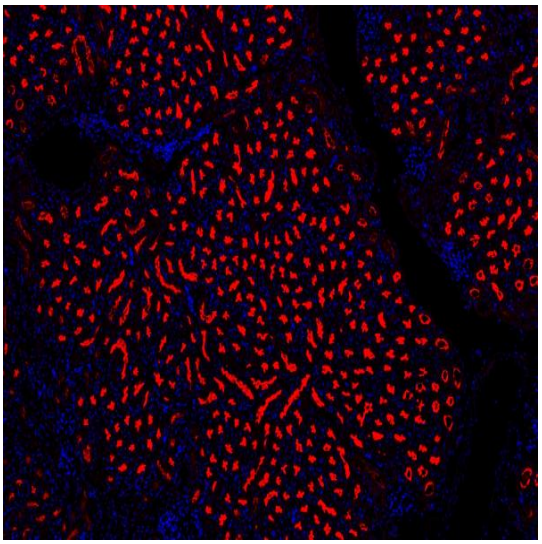

Figure 7. IF analysis of CIAS1/NALP3 using anti- CIAS1/NALP3 antibody (PA1665)
CIAS1/NALP3 was detected in paraffin-embedded section of rat kidney tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 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.

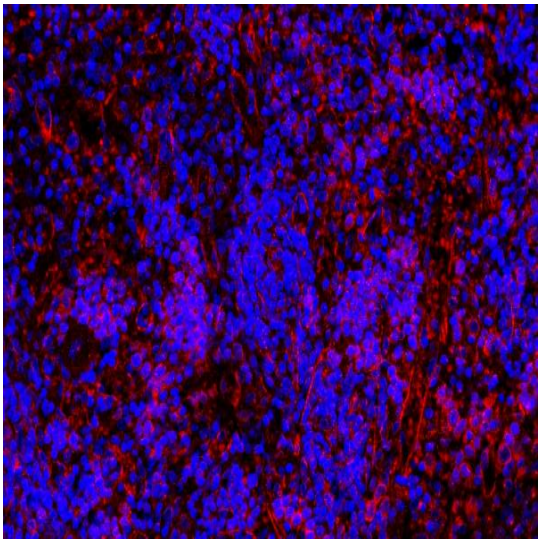

Figure 8. IF analysis of CIAS1/NALP3 using anti- CIAS1/NALP3 antibody (PA1665)
CIAS1/NALP3 was detected in paraffin-embedded section of rat spleen tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 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.

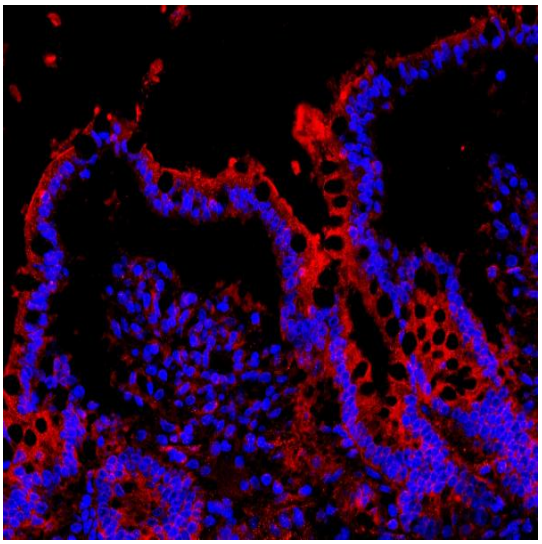

Figure 10. IF analysis of CIAS1/NALP3 using anti- CIAS1/NALP3 antibody (PA1665)
CIAS1/NALP3 was detected in paraffin-embedded section of rat colon tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 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 filter sets appropriate for the label used.

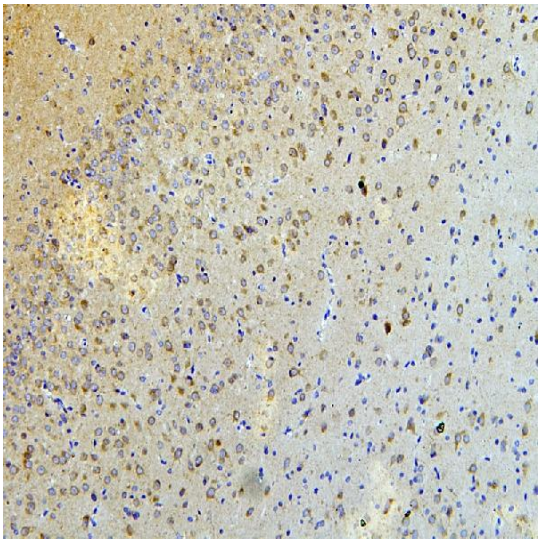

Figure 11. IHC analysis of NLRP3 using anti- NLRP3 antibody (PA1665).
NLRP3 was detected in paraffin-embedded section of rat brain tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. 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.

Approved by:

Ms. Yangjing Yue Chief technician

Quality Control System of ELISA

Date: May 13, 2016