Anti-NLRP3 Antibody [SC06-23]

ET1610-93

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 118 kDa
Clone number:	SC06-23

Description: NLRP3 is expressed predominantly in macrophages and as a component of the inflammasome, detects products of damaged cells such as extracellular ATP and crystalline uric acid. Activated NLRP3 in turn triggers an immune response. Mutations in the NLRP3 gene are associated with a number of organ specific autoimmune diseases. NLRP3 is a component of the innate immune system that functions as a pattern recognition receptor (PRR) that recognizes pathogen-associated molecular patterns (PAMPs). NLRP3 belongs to the NOD-like receptor (NLR) subfamily of PRRs and NLRP3 together with the adaptor ASC protein PYCARD forms a caspase-1 activating complex known as the NLRP3 inflammasome. NLRP3 in the absence of activating signal is kept in an inactive state complexed with HSP90 and SGT1 in the cytoplasm. NLRP3 inflammasome detects danger signals such as crystalline uric acid and extracellular ATP released by damaged cells. These signals release HSP90 and SGT1 from and recruit ASC protein and caspase-1 to the inflammasome complex. Caspase-1 within the activated NLRP3 inflammasome complex in turn activates the inflammatory cytokine, IL-1β. The NLRP3 inflammasome appears to be activated by changes in intracellular potassium caused by potassium efflux from mechanosensitive ion channels located in the cell membrane

Immunogen: Recombinant protein within Human NLRP3 aa 5-161 / 1036.

Positive control: THP-1 cell lysate, RAW264.7 cell lysate, human lung tissue lysates, HUVEC, RAW264.7, human colon carcinoma tissue, human liver carcinoma tissue, mouse bladder tissue, mouse spleen tissue, human lung carcinoma tissue, Jurkat.

Subcellular location: Cytoplasm, Inflammasome, Secreted, Nucleus, Endoplasmic reticulum.

Database links: SwissProt: Q96P20 Human | Q8R4B8 Mouse
Entrez Gene: 287362 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:100-1:500
IHC-P	1:100-1:400
FC	1:500-1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

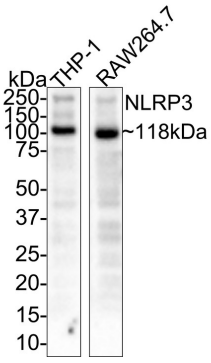

Fig1: Western blot analysis of NLRP3 on different lysates with Rabbit anti-NLRP3 antibody (ET1610-93) at 1/1,000 dilution.

Lane 1: THP-1 cell lysate
Lane 2: RAW264.7 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 118 kDa
Observed band size: 118 kDa

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1610-93) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

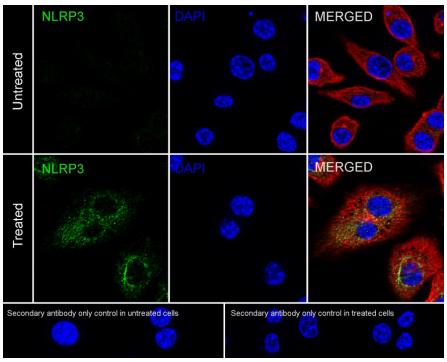

Fig2: Immunocytochemistry analysis of RAW264.7 cells treated with 10µg/mL LPS for 8 hours labeling NLRP3 with Rabbit anti-NLRP3 antibody (ET1610-93) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-NLRP3 antibody (ET1610-93) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

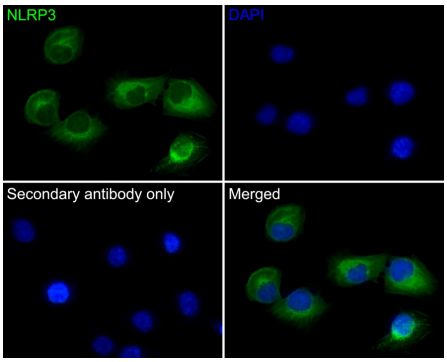

Fig3: Immunocytochemistry analysis of HUVEC cells labeling NLRP3 with Rabbit anti-NLRP3 antibody (ET1610-93) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-NLRP3 antibody (ET1610-93) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

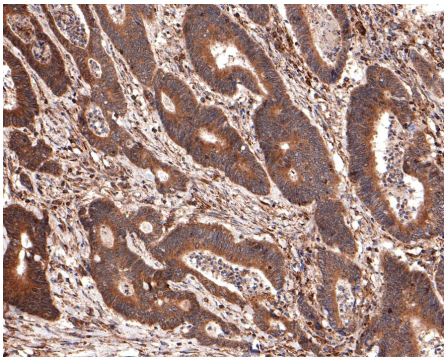

Fig4: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-NLRP3 antibody (ET1610-93) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-93) at 1/400 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

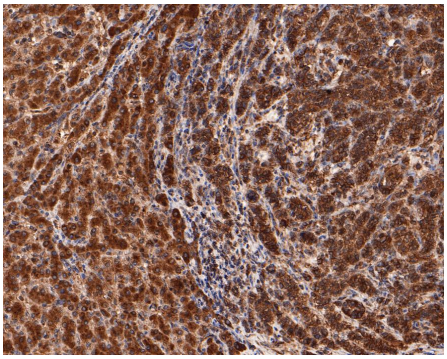

Fig5: Immunohistochemical analysis of paraffin-embedded human liver carcinoma tissue with Rabbit anti-NLRP3 antibody (ET1610-93) at 1/100 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-93) at 1/100 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Background References

1. Qiao J et al. Busulfan and cyclosphamide induce liver inflammation through NLRP3 activation in mice after hematopoietic stem cell transplantation. Sci Rep 5:17828 (2015).
2. Ataide MA et al. Malaria-induced NLRP12/NLRP3-dependent caspase-1 activation mediates inflammation and hypersensitivity to bacterial superinfection. PLoS Pathog 10:e1003885 (2014).