Anti-Human NLRP3 Antibody [PSH11-07] - BSA and Azide free (Capture)

HA723275

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	ELISA(Cap)
Clone number:	PSH11-07

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 1-218.
Positive control:	Recombinant Human NLRP3 protein.
Subcellular location:	Cytoplasm, Inflammasome, Secreted, Nucleus, Endoplasmic reticulum.
Database links:	SwissProt: Q96P20 Human
Recommended Dilutions:	
ELISA(Cap)	Use at an assay dependent concentration. Can be paired for Sandwich ELISA with Rabbit monoclonal [PSH11-08] to Human NLRP3 antibody (Detector) (HA723276) or Rabbit monoclonal [PSH11-09] to Human NLRP3 antibody (Detector) (HA723278) and Recombinant Human NLRP3 protein as the standard. The reference range value is 78-10,000 pg/ml.
Storage Buffer:	PBS (pH7.4).
Storage Instruction:	Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

Images

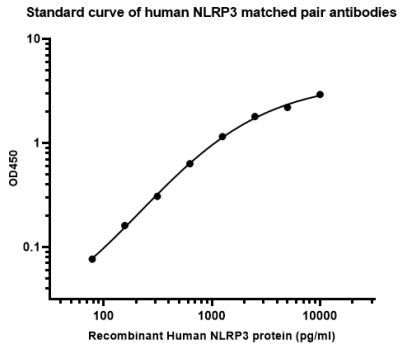

Fig1: Sandwich ELISA analysis of human NLRP3 matched pair antibodies

Elisa assay was performed by coating wells of a 96-well plate with 100 µl per well of capture antibody (HA723275) diluted in carbonate/bicarbonate buffer, at a concentration of 2ug/ml overnight at 4℃. Wells of the plate were washed, blocked with 150 µl 0.05% tween-20 1% BSA blocking buffer, and incubated with serial diluted Recombinant Human NLRP3 protein starting from 10000 pg/ml to 0 pg/ml and detect antibody (HA723276, Biotin, 0.2 µg/ml) for 1 hour at 30℃ with shaking. Then the plate was washed and incubated with 100 µl per well of SA-HRP for 0.5 hour at 30℃ with shaking. Detection was performed using an Ultra TMB Substrate for 10 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.

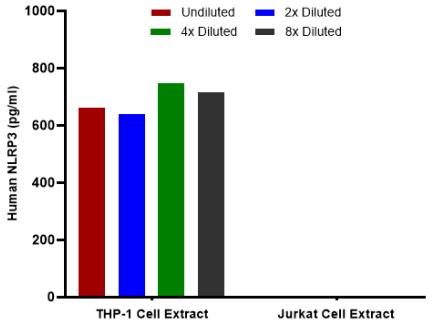

Fig2: Interpolated concentrations of native NLRP3 in THP-1 and Jurkat extract samples based on a 1000 µg/ml extract load.

Interpolated concentration of native NLRP3 was measured in duplicate at different sample concentrations and interpolated from the NLRP3 standard curves. The mean NLRP3 concentration was determined to be 691 pg/mL in THP-1 cell extract. There was no detectable signal in Jurkat cell extract.

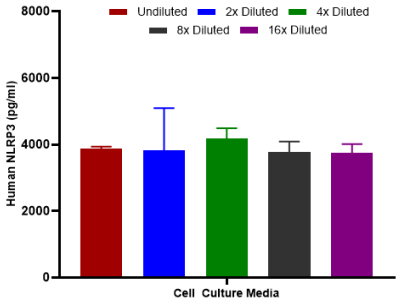

Fig3: Interpolated concentrations of spiked NLRP3 in cell culture media samples.

The concentrations of NLRP3 were measured in duplicates, interpolated from the NLRP3 standard curves and corrected for sample dilution. Undiluted samples are as follows: cell culture media 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2).

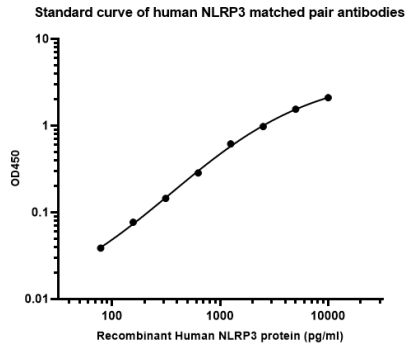

Fig4: Sandwich ELISA analysis of human NLRP3 matched pair antibodies

Elisa assay was performed by coating wells of a 96-well plate with 100 µl per well of capture antibody (HA723275) diluted in carbonate/bicarbonate buffer, at a concentration of 2ug/ml overnight at 4℃. Wells of the plate were washed, blocked with 150 µl 0.05% tween-20 1% BSA blocking buffer, and incubated with serial diluted Recombinant Human NLRP3 protein starting from 10000 pg/ml to 0 pg/ml and detect antibody (HA723278, Biotin, 0.2 µg/ml) for 1 hour at 30℃ with shaking. Then the plate was washed and incubated with 100 µl per well of SA-HRP for 0.5 hour at 30℃ with shaking. Detection was performed using an Ultra TMB Substrate for 10 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.

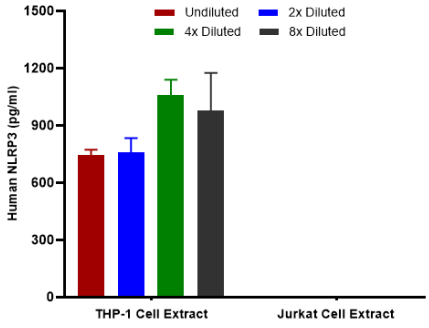

Fig5: Interpolated concentrations of native NLRP3 in THP-1 and Jurkat extract samples based on a 1000 µg/ml extract load.

Interpolated concentration of native NLRP3 was measured in duplicate at different sample concentrations and interpolated from the NLRP3 standard curves. The interpolated dilution factor corrected values were plotted (mean +/- SD, n=2). The mean NLRP3 concentration was determined to be 888 pg/mL in THP-1 cell extract. There was no detectable signal in Jurkat cell extract.

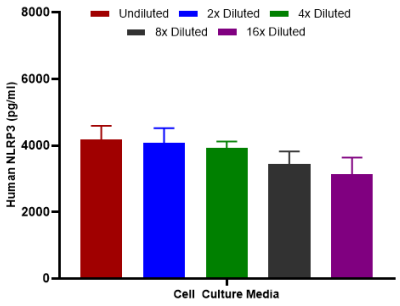

Fig6: Interpolated concentrations of spiked NLRP3 in cell culture media samples.

The concentrations of NLRP3 were measured in duplicates, interpolated from the NLRP3 standard curves and corrected for sample dilution. Undiluted samples are as follows: cell culture media 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Tengesdal IW et al. NLRP3 and cancer: Pathogenesis and therapeutic opportunities. Pharmacol Ther. 2023 Nov
2. Yu T et al. NLRP3 Cys126 palmitoylation by ZDHHC7 promotes inflammasome activation. Cell Rep. 2024 Apr