

REFERENCES

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Human NLRP3 Immunoassay

Catalog Number:SEKH-0504

For the quantitative determination of human NLRP3 concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of NLRP3 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

The linearity of the assay

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	103	104
	Range(%)	93–111	92–113
1:4	Average% of Expected	98	102
	Range(%)	87–110	91–112

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 78 pg/mL

SPECIFICITY: This assay recognizes both natural and recombinant human NLRP3. The factors listed below were prepared at 100 ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Recombinant rat
ApoAI		
BMP1		
CRP		
HGF		
TNF- α		
TGF β 1		
IL-1 α		
TLR1		
PDGF		
prolactin		

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY: The recovery of NLRP3 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of NLRP3 in two matrices

Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	94	86–99
Cell culture supernatants	103	96–117

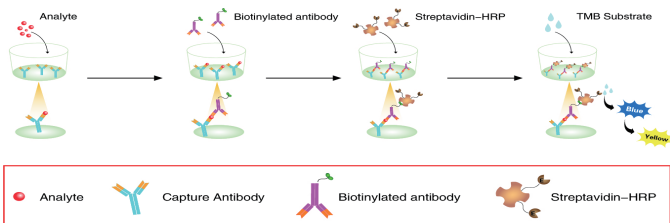
BACKGROUND

NACHT, LRR and PYD domains-containing protein 3 (NALP3), also known as cryopyrin, is a protein that in humans is encoded by the NLRP3 gene. NALP3 is expressed predominantly in macrophages and its activation triggers an immune response. Mutations in the NLRP3 gene are associated with a number of organ specific autoimmune diseases such as CAPS, FCAS, MWS, CINCA, NOMID. NALP3 is a component of the innate immune system that functions as a pattern recognition receptor (PRR) that recognizes pathogen-associated molecular patterns (PAMPs). NALP3 belongs to the NOD-like receptor (NLR) subfamily of PRRs and NALP3 together with the adaptor ASC protein PYCARD forms a caspase-1 activating complex known as the NALP3 inflammasome. NALP3 in the absence of activating signal is kept in an inactive state complexed with HSP90 and SGT1 in the cytoplasm. NALP3 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 NALP3 inflammasome complex in turn activates the inflammatory cytokine, IL-1 β .

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for NLRP3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells; any NLRP3 present is captured by the coated antibody after incubation. After washing away any unbound substances, a biotin-conjugate antibody specific for NLRP3 is added to detect the captured NLRP3 protein in the sample. Following a wash to remove any unbound combination, horseradish peroxidase (HRP)-conjugated Streptavidin is added to the wells. After extensive washing, a tetramethyl-benzidine (TMB) reagent is added to the wells for signal development. Solution containing sulfuric acid is used to stop color development. The color intensity, proportional to the quantity of bound protein, is then measurable at 450nm.

DESCRIPTION



TECHNICAL HINTS AND LIMITATIONS

1. This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

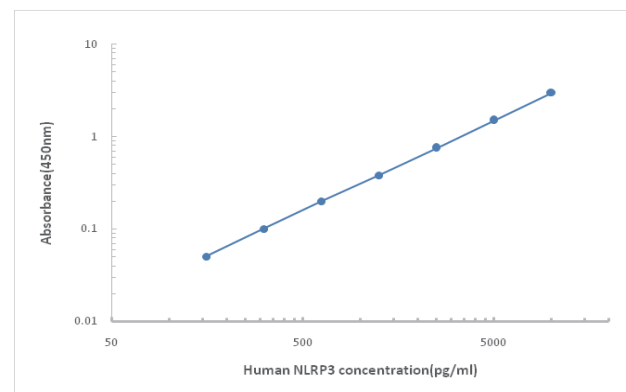
DESCRIPTION

regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the NLRP3 ELISA

Standardized (pg/ml)	OD.	OD.	Average	Corrected
0	0.048	0.044	0.046	-
156.25	0.141	0.125	0.133	0.087
312.5	0.206	0.182	0.194	0.148
625	0.306	0.271	0.288	0.242
1250	0.486	0.430	0.458	0.412
2500	0.789	0.698	0.743	0.697
5000	1.285	1.136	1.210	1.164
10000	2.093	1.851	1.972	1.926



Representative standard curve for NLRP3 ELISA.

ASSAY PROCEDURE

Prepare all reagents and standards as directed, wash the plate 3 times before the assay.



Add 100µl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 120 minutes at room temperature(25±2°C).



Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-human NLRP3 antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2°C).



Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, shaking with Micro-oscillator (100r/min) to incubate 30 minutes at room temperature (25±2°C).



Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 10-30 minutes (depending on signal) at room temperature(25±2°C). Protect from light.



Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the NLRP3 concentrations versus the log of the O.D. and the best fit line can be determined by

KIT COMPONENTS & STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells x12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8°C**
Standard - lyophilized, 10000 pg/ml upon reconstitution	2 vials	Store at 2-8°C** for six months
Concentrated Biotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C ***for six months
Concentrated Streptavidin-HRP solution(100X) - 120 ul/vial	1 vial	Store at 2-8°C ***for six months
Standard /sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C ***for six months
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C ***for six months
Streptavidin-HRP Diluent - 16 ml/vial	1 bottle	Store at 2-8°C ***for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C ***for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C ***for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C ***for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirrt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000g (or 3000rpm) to remove debris. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at $2-8^{\circ}\text{C}$. Centrifuge approximately for 15 minutes at 1000g (or 3000rpm). Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000g (or 3000rpm) within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature ($20-25^{\circ}\text{C}$) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard\Sample** - Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 10000pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500uL of Standard/Sample Diluent into 5000pg/ml tube and the remaining tubes. Use the stock solution of 10000pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 10000pg/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).

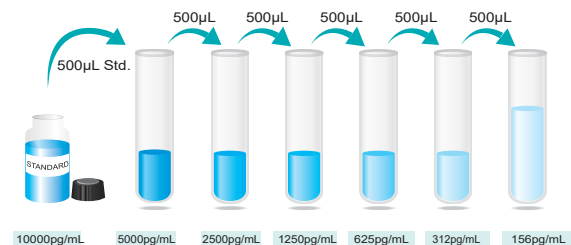
***If you do not run out of re-melting standard, store it at -20°C . Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-human NLRP3 antibody:** Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

***The working solution should be used within one day after dilution.**

5. **Working solution of Streptavidin-HRP:** Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

***The working solution should be used within one day after dilution.**



Preparation of NLRP3 standard dilutions