

REFERENCES

1. Turunen JA, et al. (2018). American Journal of Ophthalmology. 184: 41–50.
2. Martinon F (2008). Journal of Leukocyte Biology. 83 (3): 507–11.
3. Hari A, et al. (2014). Scientific Reports. 4: 7281. doi:10.1038/srep07281.
4. Haneklaus M, et al. (2013). Current Opinion in Immunology. 25 (1): 40–45.
5. Levy M, et al. (2015). 21 (3): 213–215.

Mouse NLRP3 Immunoassay

Catalog Number:SEKM-0179

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

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

Country | Company: China | Beijing Solarbio Science & Technology Co.,Ltd
Address:NO.85A, Liandong U Valley, Tongzhou District, Beijing, P.R.China.
Tel: 86-10-56371241 Fax: 86-10-56371282 E-mail: service@solarbio.com

TABLE OF CONTENTS

SECTION	PAGE
BACKGROUND.....	01
PRINCIPLE OF THE ASSAY.....	01
TECHNICAL HINTS AND LIMITATIONS.....	02
PRECAUTIONS.....	02
KIT COMPONENTS& STORAGE CONDITIONS.....	03
OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED.....	04
SPECIMEN COLLECTION & STORAGE.....	04
REAGENTS PREPARATION.....	04
ASSAY PROCEDURE.....	06
CALCULATION OF RESULTS.....	07
PERFORMANCE CHARACTERISTICS.....	09
REFERENCES.....	10

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 78.13pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant mouse NLRP3. The factors listed below were prepared at 10ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

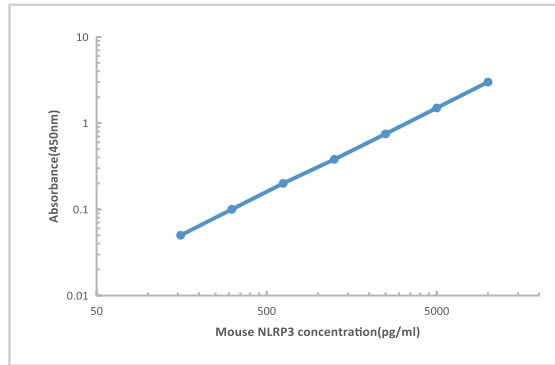
Factors assayed for cross-reactivity

Recombinant mouse	Recombinant human	Recombinant porcine
BMP1	NLRP3	
BMP2		
BMP4		
IL-1 β		
IL-4		
IL-5		
IL-6		
IL-8		
IFN- γ		

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

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

Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	99	87–110
Cell culture supernatants	107	95–119



Representative standard curve for NLRP3 ELISA

BACKGROUND

NLRP3, also known as NALP3, CIAS1, PYPAF or Cryopyrin, is a cytosolic ~120 kDa member of the NLRP family of proteins expressed in leukocytes, especially neutrophils. As a component of the inflammasome, NLRP3 activates caspases 1 and 5. Defects in NLRP3 may cause FCAS1, CINCA, or Muckle-Wells autoinflammatory syndromes. Mouse NLRP3 contains an N-terminal Pyrin domain (aa 1-91) followed by a Nacht region (aa 216-532), and seven LRRs (aa 739-988). Within the sequence used as an immunogen, mouse NLRP3 shares 78% and 93% aa identity with human and rat NLRP3, respectively. Alternate splicing of mouse NLRP3 generates additional isoforms that lack either LRR2 and 3, LRR 6 and 7, or LRR4-9.

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 and any NLRP3 present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for NLRP3 is added to detect the captured NLRP3 protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by Tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.



TECHNICAL HINTS AND LIMITATIONS

1. This Solarbio ELISA should not be used beyond the expiration date 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.

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 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.

Standardized(pg/ml)	OD.	OD.	Average	Corrected
0	0.049	0.048	0.049	-
156.25	0.162	0.151	0.156	0.107
312.5	0.235	0.219	0.227	0.178
625	0.350	0.326	0.338	0.289
1250	0.556	0.517	0.536	0.488
2500	0.902	0.840	0.871	0.822
5000	1.469	1.368	1.418	1.370
10000	2.393	2.228	2.311	2.262

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-Mouse CTNT 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 5-30 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.

KIT COMPONENTS & STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells × 12 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. Squirr 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 at 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.

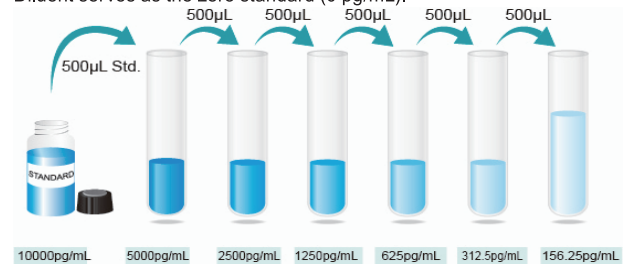
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000g (or 3000rpm) within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Sample dilution: Samples should be diluted with four volumes of 1 x Assay Buffer and vortex for 1 min prior to assay. If the OD value still exceeds the upper limit of the standard curve, further dilution is recommended till it falls in the detection range and the dilution factor must be used for calculation of the concentration.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature ($20-25^{\circ}\text{C}$) before use.
2. **Assay Buffer (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/Specimen:** Add 1ML of Standard/Sample Diluent to a Standard vial to make the high standard concentration of 10000pg/ml. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500 μL 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 10000 pg/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).



Preparation of NLRP3 standard dilutions

***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-mouse 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.**