THP1-KO-NLRP3 Cells

NLRP3 knockout human monocytes

Catalog code: thp-konlrp3

https://www.invivogen.com/thp1-ko-kd-nlrp3

For research use only

Version 20A13-ED

PRODUCT INFORMATION

Contents

- 3-7 x 10^6 THP1-KO-NLRP3 cells in a cryovial or shipping flask

IMPORTANT: If cells provided in a cryovial are not frozen upon arrival, contact InvivoGen immediately.

- 1 ml of Normocin™ (50 mg/ml). Normocin™ is a formulation of three antibiotics active against mycoplasmas, bacteria, and fungi. Store at -20 °C.*

*The expiry date is specified on the product label.

Note: Data sheets for all components are available on our website.

Handling of Frozen Cells Upon Arrival

Cells must be thawed immediately upon receipt and grown according to handling procedures (as described on the next page) to ensure the best cell viability and proper assay performance.

*Note: **Avoid freezing cells upon receipt** as it may result in irreversible damage to the cell line.*

Disclaimer: We cannot guarantee cell viability if the cells are not thawed immediately upon receipt and grown according to handling procedures.

IMPORTANT: For cells that arrive in a shipping flask please refer to the enclosed 'cell recovery procedure'.

Cell Line Stability

Genetic instability is a biological phenomenon that occurs in all stably transfected cells, resulting in reduced responsiveness in normal cell culture conditions. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages.

Quality Control

- Biallelic *NLRP3* knockout has been verified by DNA sequencing, PCR, Western blot (WES™), and functional assays.
- The stability for 20 passages, following thawing, has been verified.
- These cells are guaranteed mycoplasma-free.

SAFETY CONSIDERATIONS

Biosafety Level 1

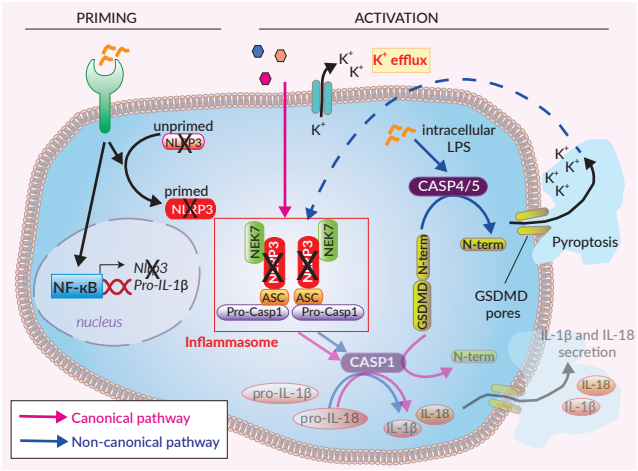
PRODUCT DESCRIPTION

THP1-KO-NLRP3 cells were generated from the human monocytic THP1 cell line through the stable knockout of the *NLRP3* gene, causing the loss of NLRP3 expression. In THP1-KO-NLRP3 cells, upon canonical inflammasome activation by inducers such as Nigericin and MSU crystals, mature IL-1β secretion and pyroptotic cell death are abolished. Moreover, upon non-canonical inflammasome activation by inducers such as outer membrane vesicles (*E. coli* OMVs) and transfected lipopolysaccharide (LPS), the NLRP3-caspase-1 dependent maturation of IL-1β is abolished, but pyroptosis still occurs through the activation of caspase-4/5, and subsequent cleavage of gasdermin D.

BACKGROUND

Inflammasomes are cytoplasmic multi-protein complexes, characterized by a primary sensor, that assemble in response to infections and cellular damage. NLRP3 (NOD-like receptor pyrin domain-containing protein 3, cryopyrin or NALP3) is the best characterized inflammasome sensor.

The inflammasome response requires two signals, priming (recognition of PAMPs or DAMPs by pattern recognition receptors such as TLRs) and activation[1,2]. Activation of NLRP3 can be induced by a wide range of stimuli (e.g. Nigericin, ATP, crystals), and instead of directly binding to them, NLRP3 senses downstream cytosolic stress signals such as ion imbalances (i.e. K+ efflux)[1,2]. The canonical inflammasome response is driven by aggregation of the sensor, NLRP3, with the ASC adaptor and pro-caspase-1. Activation of caspase-1 (CASP1) induces the maturation of pro-IL-1β/pro-IL-18 and cleavage of the pore-forming gasdermin D (GSDMD), leading to the secretion of IL-1β/-18 and pyroptosis[1,2]. Additionally, NLRP3 is activated indirectly by the induction of the non-canonical inflammasome (CASP4/5 in humans and CASP11 in mice) upon the sensing of cytosolic LPS. These caspases trigger GSDMD-driven release of alarmins and K^+ efflux, which ultimately induces the activation of NLRP3 and CASP1-mediated IL-1β/-18 maturation and secretion[1,2].



1. Swanson K.V. *et al.*, 2019. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nat. Rev. Immunol. 19:477. **2. Groslambert M. & Py B. 2018**. Spotlight on the NLRP3 inflammasome pathway. J. Inflamm. Res. 11:359.

USE RESTRICTIONS

These cells are distributed for research purposes only.

This product is covered by a Limited Use License. For non-research use, such as screening, quality control or clinical development, please contact info@invivogen.com.

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com

InvivoGen
www.invivogen.com

HANDLING PROCEDURES

Required Cell Culture Medium

• **Growth Medium:** RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml Normocin™

Initial culture of all THP1-derived cells must be performed in growth medium containing 20% heat-inactivated FBS.

Note: The use of Normocin™ together with Pen-Strep is required to keep the cells free of microbial contaminants. Contamination of this cell line may activate PRRs, such as TLRs, resulting in differentiation of the monocytes and activation of PRR signaling pathways.

• **Test Medium:** RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin

• **Freezing Medium:** 95% FBS and 5% DMSO

Initial Culture Procedure

The first propagation of cells should be for generating stocks for future use. This ensures the stability and performance of the cells for subsequent experiments.

1. Thaw the vial by gentle agitation in a 37 °C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid.
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol.

Note: All steps from this point should be carried out under strict aseptic conditions.

3. Transfer cells to a larger tube containing 15 ml of pre-warmed growth medium (with 20% heat-inactivated FBS).
4. Centrifuge cells at 150 x g (RCF) for 10 mins.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium (with 20% heat-inactivated FBS).
6. Transfer the cells to a T-25 culture flask containing 5 ml of growth medium (with 20% heat-inactivated FBS).
7. Place the culture at 37 °C in 5% CO_2.

Cell Maintenance

1. THP1-KO-NLRP3 cells grow in suspension.
2. After cells have recovered and are growing well (following at least two passages), maintain and subculture the cells in growth medium.
3. Pass the cells every 3 days by inoculating 5 x 10^5 cells/ml. Do not allow the cell concentration to exceed 2 x 10^6 cells/ml.

Note: The average doubling time for the THP1-KO-NLRP3 cells is ~72 hours using the conditions described above.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10^6 cells/ml in freshly prepared freezing medium with cold FBS.

Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.

2. Dispense 1 ml of the cell suspension into cryogenic vials.
3. Place vials in a freezing container and store at -80 °C overnight.
4. Transfer vials to liquid nitrogen for long term storage.

Note: If properly stored, cells should remain stable for years.

Cell Handling Recommendations

To ensure the best results, use THP1-KO-NLRP3 cells with less than 20 passages.

EXPERIMENTAL PROCEDURES

THP1-KO-NLRP3 cells are designed to study the signals involved in inflammasome activation. Below is an example protocol to induce canonical and non-canonical inflammasome responses.

Cell preparation

1. The day prior the assay, pass cells at 5 x 10^5 cells/ml in growth medium.
2. On the day of the assay, centrifuge at 150 x g (RCF) for 10 mins or 300 x g (RCF) for 5 mins.
3. Remove supernatant and resuspend THP1-KO-NLRP3 cells at 1.6 x 10^6 cells/ml in freshly prepared, pre-warmed **test medium.**

Priming

1. Dispense 20 µl of LPS-EK at 10 µg/ml (final concentration: 1 µg/ml) per well of a flat-bottom 96-well plate.
2. Add 180 µl of cell suspension (~300,000 cells) per well.
3. Incubate the plate for 3h at 37 °C in 5% CO_2.

Activation

1. Carefully remove culture supernatant . Add 180 µl of test medium.
2. Add 20 µl of an inflammasome inducer per well.

Note: We recommend to perform a dilution series for each inducer (e.g. 1:2 dilution series of Nigericin starting at 10 µM).

3. Include a negative control (no inducer).
4. Incubate the plate for 6h at 37°C in 5% CO_2.
5. Take 100 µl of culture supernatant for analysis of human (h)IL-1β secretion and/or cell death.

Optional: These samples can be stored at -80°C until required.

6. Add 100 µl of test medium to each well of the original culture plate and continue to incubate for an additional 18h at 37°C in 5% CO_2.
7. Take 100 µl of culture supernatant for analysis of hIL-1β secretion and/or cell death.

Optional: These samples can be stored at -80°C until required.

Detection of mature hIL-1β and cell death in supernatant

• The secretion of bioactive hIL-1β in the supernatant of THP-1 KO-NLRP3 cells can be assessed using InvivoGen's HEK-Blue™ IL-1β sensor cells. For more details on how to use these cells please visit https://www.invivogen.com/hek-blue-il1b

• Cell death can be monitered using classical assays such as the lactate dehydrogenase (LDH) assay, following the manufacturer's instructions.

RELATED PRODUCTS

Product	Description	Cat. Code
MSU Crystals	Inflammasome inducer	tlrl-msu
Nigericin	Inflammasome inducer	tlrl-nig
E. coli OMVs	Inflammasome inducer	tlrl-omv-1
Recominant hIFN-γ	Recombinant cytokine	rcyec-hinfg
LPS-EK (*E. coli* K12)	TLR4 agonist	tlrl-peklps
HEK-Blue™ IL-1β cells	IL-1β reporter cells	hkb-il1b
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs1

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com