THP1-KO-NLRC4 Cells

NLRC4 knockout human monocytes

Catalog code: thp-konlrc4z

https://www.invivogen.com/thp1-konlrc4

For research use only

Version 21A11-NJ

PRODUCT INFORMATION

Contents

- 3-7 x 10^6 THP1-KO-NLRC4 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 to prevent contamination from mycoplasmas, bacteria, and fungi. Store at -20 °C.*
- 1 ml of Zeocin™ (100 mg/ml). Store at 4°C or-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.
- THP1-KO-NLRC4 cells should not be passaged more than 20 times to remain fully efficient. These cells should be maintained in growth medium supplemented with the selective antibiotic Zeocin™ (100 µg/ml) following every other passage.

Quality Control

- Biallelic knockout encompassing the NBD (nucleotide-binding domain of *NLRC4* 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.

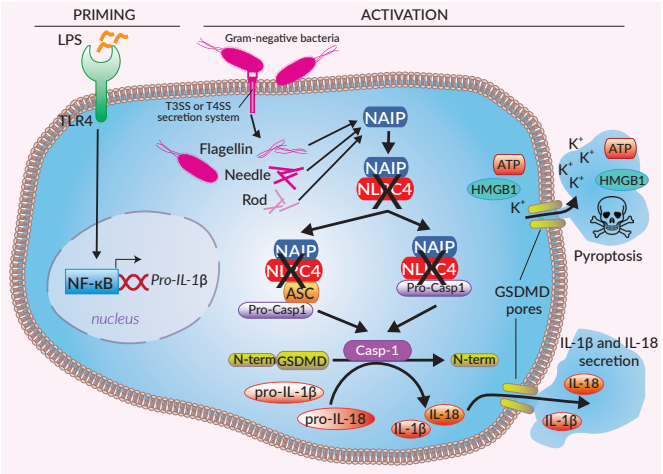
PRODUCT DESCRIPTION

THP1-KO-NLRC4 cells were generated from the human monocytic THP1-Null2 cell line through a deletion encompassing the nucleotide-binding domain (NBD) in the gene encoding *NLRC4*. NBD is essential for NLRC4 polymerization and inflammasome assembly[1]. Thus THP1-KO-NLRC4 cells express an inactive NLRC4 protein, unable to form a functional inflammasome complex. Mature IL-1β secretion and pyroptosis are therefore abolished in these cells following activation of the NLRC4 inflammasome (e.g. LFn-Needle).

THP1-KO-NLRC4 cells are resistant to Zeocin™.

BACKGROUND

Inflammasomes are cytoplasmic multi-protein complexes, characterized by a primary sensor, that assemble in response to infections and cellular damage. NLRC4 (Nucleotide-binding domain (NBD) and leucin-rich repeat (LRR) receptor, CARD domain-containing protein 4, or IPAF) senses intracellular bacterial molecules such as Flagellin from the motility apparatus, or Inner Rod, and Needle proteins from the type III or IV secretion systems (T3SS or T4SS). More specifically, NLRC4 associates with NAIP (NLR family apoptosis inhibitory protein) which directly binds to the ligands. In humans, a single NAIP operates upstream of NLRC4 and binds to each of the above-mentionned activators[1-2]. Once recruited by NAIP, NLRC4 triggers homo-polymerization, resulting in the clustering of NLRC4 CARD domains. The NLRC4/NAIP complex then associates with pro-caspase-1, either via direct CARD-CARD interactions, and/or through the ASC (apoptosis-associated speck-like protein) adaptor[1]. Activation of caspase-1 induces the cleavage of pro-IL-1β/pro-IL-18, formation of gasdermin D (GSDMD) pores, secretion of IL-1β/-18, and pyroptosis[1-3]. The NLRC4 inflammasome appears to protect mucosal barriers such as the lung, stomach, and intestine from invading bacteria[3]. Gain-of-function mutations have been described in human NLRC4 and are associated with auto-inflammatory conditions[3].



1. Zhang L. *et al.*, 2015. Cryo-EM structure of the activated NAIP2-NLRC4 inflammasome reveals nucleated polymerization. Science. 350:404-409. **2. Reyes Ruiz, V.M. *et al.*, 2017.** Broad detection of bacterial type III secretion system and flagellin proteins by the human NAIP/NLRC4 inflammasome. PNAS 114(50):13242-13247. **3. Bauer R. & Rauch I., 2020**. The NAIP/NLRC4 inflammasome in infection and pathology. Mol. Aspects Med. 2020 Jun 1:100863.

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
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FAQ Any questions about our cell lines? Visit our FAQ page.

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SAFETY CONSIDERATIONS

Biosafety Level 1

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, **without Normocin™ and Zeocin™**

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

• **Required selective antibiotic:** Zeocin™

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 at RCF 150 g for 10 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium (with 20% heat-inactivated FBS). **Do not add selective antibiotics until the cells have been passaged twice.**
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-NLRC4 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. To maintain selection pressure, add 100 µg/ml of Zeocin™ to the growth medium every other passage.
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-NLRC4 cells is ~67 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-NLRC4 cells with less than 20 passages.

EXPERIMENTAL PROCEDURES

THP1-KO-NLRC4 cells are designed to study the signals involved in inflammasome activation. Below is an example protocol to induce canonical and non-canonical inflammasome responses.

It is recommended to perform assays with test medium which does not contain Normocin™ and Zeocin™.

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 cells at RCF 300 g for 5 minutes.
3. Remove supernatant and resuspend THP1-KO-NLRC4 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 inflammasome inducer per well.

Note: We recommend to perform a dilution series for each inducer (e.g. 1:5 dilution series of LFn-Needle starting at 100 ng/ml final concentration. LFn-Needle must be used in combination with the anthrax protective antigen. Please refer to the LFn-Needle technical data sheet.)

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 THP1-KO-NLRC4 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
Zeocin™	Selection antibiotic	ant-zn-1
LFn-Needle	Inflammasome inducer	tlrl-ndl
Nigericin	Inflammasome inducer	tlrl-nig
Poly(dA:dT)	Inflammasome inducer	tlrl-patn
LPS-EK (*E. coli* K12)	TLR4 agonist	tlrl-peklps
HEK-Blue™ IL-1β cells	IL-1β reporter cells	hkb-il1bv2
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs1
THP1-Null2 cells	Human THP-1 monocytes	thp-nullz

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