THP1-NLRC4 Cells

NLRC4 inflammasome test cell line

Catalog code: thp-nlrc4

https://www.invivogen.com/thp1-nlrc4

For research use only

Version 23E29-MM

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10^6 THP1-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 Blasticidin (10 mg/ml). Store at 4 °C or at -20 °C.*
- 1 ml Normocin™ (50 mg/ml). Normocin™ is a formulation of three antibiotics to prevent contamination from mycoplasma, 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 Cells Upon Arrival

Cells must be thawed immediately upon receipt and grown according to handling procedures (as decribed on the next page) to ensure the best cell viability and 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-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 Blasticidin (10 µg/ml) following every other passage.

Quality control

- Expression of NLRC4 was confirmed by qRT-PCR and functional assays.
- The stability for 20 passages, following thawing, has been verified.
- These cells are guaranteed mycoplasma-free.

SAFETY CONSIDERATIONS

Biosafety Level 1

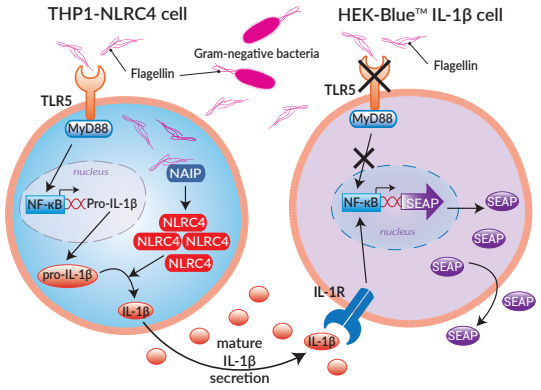
USE RESTRICTIONS

These cells are distributed for research purposes only.

This product is covered by a Limited Use License. By use of this product, the buyer agrees to the terms and conditions of all applicable Limited Use Label Licenses. For non-research use, such as screening, quality control or clinical development, contact info@invivogen.com.

PRODUCT DESCRIPTION

THP1-NLRC4 cells are derived from the THP-1 human monocytic cell line. They stably overexpress NLRC4 and naturally express TLR5. NLRC4 and TLR5 are two pattern recognition receptors for bacterial Flagellins in the cytosol and in the extracellular milieu, respectively. Substantial Flagellin amounts are required for intracellular detection by NLRC4/NAIP inflammasome in THP-1 cells. NLRC4 overexpression increases the cell line responsiveness to Flagellins without impacting the inflammasome response to highly potent NLRC4 inducers, such as LFn-Needle. Levels of IL-1β secreted into the cell supernatant can be monitored using the HEK-Blue™ IL-1β cell line. THP1-NLRC4 cells are resistant to Blasticidin.



BACKGROUND

NLRC4 (Nucleotide-binding domain (NBD) and leucine-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[1-3].

Flagellin is an agonist not only for NAIP/NLRC4, but also the surface Toll-like receptor 5 (TLR5). Extracellular flagellin is detected by TLR5, resulting in MyD88-mediated NF-κB activation and the production of proinflammatory cytokines, including pro-IL-1β. Intracellular Flagellin induces the formation of the NAIP/NLRC4 inflammasome resulting in caspase-1 activation, cleavage of pro-IL-1β, formation of gasdermin D (GSDMD) pores, IL-1β secretion, and eventually, pyroptosis[1-3].

1. Zhao Y. *et al.*, 2011. The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. Nature. 477:596-600. **2. Zhang L. *et al.*, 2015.** Cryo-EM structure of the activated NAIP2-NLRC4 inflammasome reveals nucleated polymerization. Science. 350:404-409. **3. 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.

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 Asia: +852 3622-3480
E-mail: info@invivogen.com

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

InvivoGen
www.invivogen.com

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated fetal bovine serum (FBS; 30 min at 56°C), 100 µg/ml Normocin™, Pen-Strep (100 U/ml-100 µg/ml)

Initial culture of all THP-1 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 TLRs resulting in differentiation of the monocytes and activation of the reporter gene.

- **Test Medium:** RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated FBS, Pen-Strep (100 U/ml-100 µg/ml), **without Normocin™ and Blasticidin**
- **Freezing Medium:** 95% FBS, 5% DMSO

Required selective antibiotic: Blasticidin

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 in a vial containing 15 ml of pre-warmed growth medium (with 20% heat-inactivated FBS).
4. Centrifuge vial at 150 x g (RCF) for 10 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium. **Do not add selection antibiotics until the cells have been passaged twice.**
6. Transfer the vial contents 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-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 10 µg/ml of Blasticidin 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-NLRC4 cells is ~55 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 preapred 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 Recommandations

To ensure the best results, use THP1-NLRC4 cells with less than 20 passages.

EXPERIMENTAL PROCEDURES

THP1-NLRC4 cells are designed to study the activation of the NLRC4 inflammasome by Flagellins. For more information on InvivoGen's Inflammasome Test Cells, please visit: https://www.invivogen.com/inflammasome-test-cells.

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 300 x g (RCF) for 5 minutes.
3. Remove supernatant and resuspend THP1-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 Flagellin per well, such as FLA-BS Ultrapure, 200 ng/ml.
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-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
Blasticidin	Selective antibiotic	ant-bl-1
Normocin™	Antimicrobial agent	ant-nr-1
HEK-Blue™ IL-1β cells	IL-1β reporter cells	hkb-il1bv2
LPS-EK	LPS from *E. coli* K12	tlrl-eklps
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs
NLRC4 Inflammasome inducers		
FLA-BS Ultrapure	Flagellin from *B. subtilis*	tlrl-pbsfla
FLA-PA Ultrapure	Flagellin from *P. aeruginosa*	tlrl-pafla
FLA-ST Ultrapure	Flagellin from *S. typhimurium*	tlrl-pstfla