THP1-defNLRP3 Cells

Human monocytes with reduced NLRP3 activity

Catalog code: thp-dnlp

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

For research use only

Version 19K18-MM

PRODUCT INFORMATION

Contents and Storage

- **1 vial of THP1-defNLRP3 cells** (3-7 x 10^6 cells)
- **1 ml of Hygromycin B Gold** (>90% pure hygromycin B) provided at 100 mg/ml. Store at 4 °C or at -20 °C.*
- **1 ml of Normocin™** (50 mg/ml), a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20 °C.*

*The expiry date is specified on the product label.

Handling 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.

Cell Line Stability

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

THP1-defNLRP3 cells should not be passaged more than 20 times to remain fully efficient. THP1-defNLRP3 cells should be maintained in growth medium supplemented with the selective antibiotic, Hygromycin B Gold (200 µg/ml), following every other passage.

Quality control

- The reduction in NLRP3 activity in THP1-defNLRP3 cells was confirmed by qRT-PCR and a functionality assay using inflammasome inducers.
- The stability of this cell line for 20 passages following thawing has been verified.
- THP1-defNLRP3 cells are guaranteed mycoplasma-free.

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 with 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-defNLRP3 cells are derived from THP-1 human monocytic cells. THP-1 cells are the most commonly used model cell line for the study of inflammasome activation as they express high levels of NLRP3, ASC and pro-caspase-1.

THP1-defNLRP3 cells have reduced NLRP3 activity but are proficient for ASC and caspase-1 activities. They display significantly weaker responses to inducers of the NLRP3 inflammasome, such as ATP[1] and MSU crystals (see figure 1), when compared to THP1-Null cells, a positive control cell line that is fully efficient for NLRP3 activity. THP1-defNLRP3 cells also show a greatly reduced response to extracellular calcium, a danger signal that activates the NLRP3 inflammasome[2]. However, THP1-defNLRP3 cells respond to signals that activate other ASC-dependent inflammasomes, such as NLRP1 and NLRC4 inflammasomes. THP1-defNLRP3 cells, together with THP1-Null cells, are designed as tools to study the involvement of NRLP3 in response to a given signal.

THP1-defNLRP3 cells are resistant to hygromycin B.

1. Lerner A. *et al.*, 2012. IRE1α induces thioredoxin-interacting protein to activate the NLRP3 inflammasome and promote programmed cell death under irremediable ER stress. Cell Metab. 16:250-64. **2. Rossol M. *et al.*, 2012.** Extracellular $Ca2^+$ is a danger signal activating the NLRP3 inflammasome through G protein-coupled calcium sensing receptors. Nat Commun. 3:1329.

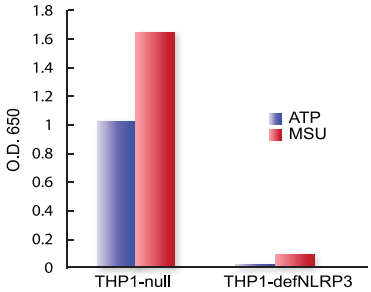

Figure 1: IL-1β production in THP1-null and THP1-defNLRP3 cells following their stimulation with ATP or MSU crystals. Cells primed with LPS (1 µg/ml) were stimulated with ATP (5 mM) or MSU crystals (100 µg/ml). After 24h, supernatants were added to HEK-Blue™ IL-1β cells. IL-1β-induced activation of NF-κB was assessed by measuring the levels of SEAP in the supernatant of HEK-Blue™ IL-1β cells using the QUANTI-Blue™ assay.

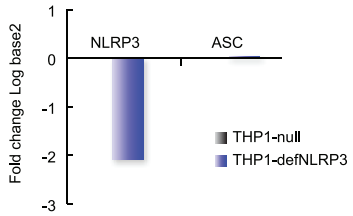

Figure 2: Quantitative RT-PCR analysis showing the fold change of NLRP3 and ASC genes in THP1-defNLRP3 cells compared to THP1-null cells.

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

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

InvivoGen
www.invivogen.com

SAFETY CONSIDERATIONS

Biosafety Level 1

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated fetal bovine serum (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.

- **Freezing Medium:** 90% fetal bovine serum (FBS), 10% DMSO
- **Test Medium:** RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated fetal bovine serum, Pen-Strep (100 U/ml-100 µg/ml)

Required Selective Antibiotic(s)

Hygromycin B

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.
4. Centrifuge vial at ~800 RPM (RCF 150 g) for 10 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium. **Do not add selective antibiotics until the cells have been passaged twice.**
6. Transfer the vial contents to a 25 cm^2 tissue culture flask containing 5 ml of growth medium.
7. Place the culture at 37 °C in 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 5 -7 x 10^6 cells/ml in freezing medium.
2. Aliquot 1 ml cells 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 Maintenance

- After cells have recovered (after at least one passage), subculture the cells in growth medium. To maintain selection pressure, add 200 µg/ml of Hygromycin B Gold to the growth medium every other passage.
- 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.

Cell Handling Recommendations

To ensure the best results:

- *Use THP1-defNLRP3 cells with less than 20 passages.*
- *Handling of cells should be as short as possible to prevent any damage resulting from the prolonged stay at room temperature without 5% CO_2.*

APPLICATION

THP1-defNLRP3 cells are designed to study the signals involved in inflammasome activation. Notably, as THP1-defNLRP3 cells express negligible levels of NLRP3, they produce minimal IL-1β in response to NLRP3 inflammasome inducers. Their response should be compared to the response of the positive control cell line THP1-Null Cells.

To become responsive to inflammasome inducers, THP1 cells must be induced by stimuli commonly used for induction in model systems, such as lipopolysaccharide (LPS) and phorbol 12-myristate acetate (PMA). Stimulation by LPS or differentiation with PMA induces the production of pro-IL-1β, the immature form of IL-1β. Subsequent stimulation with inflammasome inducers, such as ATP and MSU crystals, leads to caspase-1 activation and IL-1β maturation and secretion. Mature IL-1β can be detected by Western blot, ELISA, or a cell-based assay.

InvivoGen has developed a new method to detect bioactive IL-1β, based on HEK293 cells specifically engineered to selectively respond to IL-1β, named HEK-Blue™ IL-1β cells. These cells feature the SEAP (secreted embryonic alkaline phosphatase) reporter gene under the control of an NF-κB-inducible promoter. They naturally express the IL-1β receptor (IL-1R), and all the proteins involved in the MyD88-dependent IL-1R signaling pathway that leads to NF-κB activation. Thus upon IL-1β binding to IL-1R, a signaling cascade is initiated triggering NF-κB activation and the subsequent production of SEAP. Detection of SEAP in the supernatant of HEK-Blue™ IL-1β cells can be readily assessed using QUANTI-Blue™ Solution, a SEAP detection medium. QUANTI-Blue™ Solution turns blue in the presence of SEAP which can be easily quantified using a spectrophotometer.

Detection of IL-1β in THP-1 supernatants

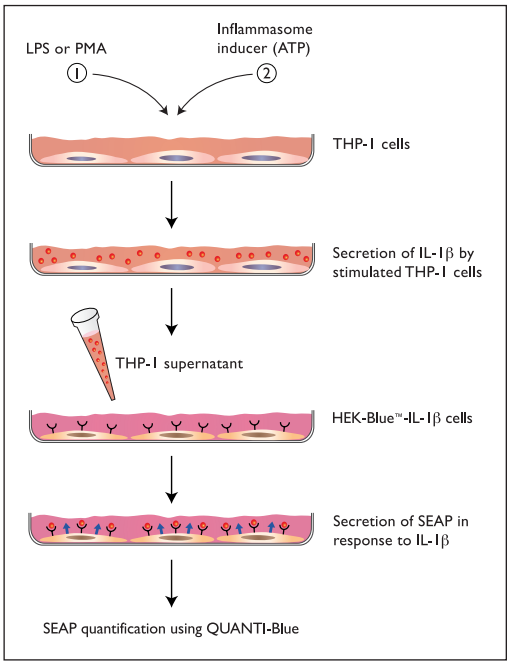

Figure 3. THP-1/HEK-Blue™ IL-1β Assay

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

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

Activation of THP1 cells

THP1 cells are grown in RPMI 1640 medium, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated fetal bovine serum (30 min at 56°C), 100 µg/ml Normocin™, Penicillin (100 U/ml), Streptomycin (100 µg/ml). THP1 cells are grown in suspension to a density of 5 x 10^5 cells/ml in tissue culture flasks.

Notes:

- Primed THP1-Null cells produce IL-1β upon stimulation with inflammasome inducers.

- As THP1-defNLRP3 cells express negligible levels of NLRP3, they produce minimal IL-1β in response to NLRP3 inflammasome inducers.

• Option 1: PMA induction

Day 1

1. Add 180 µl of THP1 cell suspension per well of a 96-well plate (2 x 10^5 cells/well).
2. Treat THP1 cells with 20 µl of PMA (final concentration 20-50 ng/ml) for 3 hours at 37 °C in 5% CO_2.
3. Gently remove medium and add 200 µl of supplemented RPMI.

Day 4

4. Wash cells with PBS and add 180 µl of supplemented RPMI.
5. Add 20 µl of an inflammasome inducer, such as ATP or MSU crystals (see Related Products).
6. Incubate overnight at 37 °C in 5% CO_2.

Note: The production of pro-IL-1β can be further increased by priming PMA-activated THP1 cells with LPS (follow protocol below).

• Option 2: LPS induction

1. Add 180 µl of THP1 cell suspension per well of a 96-well plate (3 x 10^5 cells/well).
2. Treat THP1 cells with 20 µl of LPS (final concentration 1 µg/ml) for 3 hours at 37 °C in 5% CO_2.
3. Gently remove medium and add 180 µl of supplemented RPMI.
4. Add 20 µl of an inflammasome inducer, such as ATP or MSU crystals.
5. Incubate overnight at 37 °C in 5% CO_2.

Detection of IL-1β by HEK-Blue™ IL-1β cells

HEK-Blue™ IL-1β cells are grown in DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum, 100 µg/ml Normocin™, and Pen-Strep.

Day 1

1. Prepare a HEK-Blue™ IL-1β cell suspension: wash cells with pre-warmed PBS, detach cells by tapping the flask, resuspend cells in fresh growth medium and prepare a cell suspension at 3 x 10^5 cells/ml.

Note: The response of HEK-Blue™ IL-1β cells can be altered by the action of trypsin. Do not use trypsin to detach HEK-Blue™ IL-1β cells.

2. Add 50 µl of activated THP1 cell supernatant in a well of a flat-bottom 96-well plate.
3. In separate wells, add 50 µl of recombinant human IL-1β at 0.25 µg/ml, as the positive control, and 50 µl of recombinant human TNF-α at 0.25 µg/ml, as a negative control.

Note: HEK-Blue™ IL-1β cells do not respond to human TNF-α.

4. Add 150 µl of HEK-Blue™ IL-1β cell suspension (~50,000 cells) per well.
5. Incubate overnight at 37 °C in 5% CO_2.

Day 2

6. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed product data sheet.
7. Add 180 µl of resuspended QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
8. Add 20 µl of induced HEK-Blue™ IL-1β cells supernatant.
9. Incubate the plate at 37 °C for 1-6 hours.
10. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Description	Catalog Code
ATP	Inflammasome inducer	tlrl-atp
CPPD Crystals	Inflammasome inducer	tlrl-cppd
HEK-Blue™ IL-1β	IL-1β reporter cells	hkb-il1b
Hemozoin	Inflammasome inducer	tlrl-hz
Hygromycin B Gold	Selective antibiotic	ant-hg-1
LPS-EK (*E. coli* K12)	TLR4 agonist	tlrl-eklps
MSU Crystals	Inflammasome inducer	tlrl-msu
Nigericin	Inflammasome inducer	tlrl-nig
Normocin™	Antimicrobial agent	ant-nr-1
PMA	NF-κB activator	tlrl-pma
Poly(dA:dT)/LyoVec™	Inflammasome inducer	tlrl-patc
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs
Recombinant human IL-1β	Recombinant cytokine	rcyec-hil1b
Recombinant human TNF-α	Recombinant cytokine	rcyc-htnfa
THP1-defASC	ASC deficient THP-1 cells	thp-dasc
THP1-defCASP1	CASP1 deficient THP-1 cells	thp-dcasp1
THP1-Null	Positive control cells	thp-null