THP1-Null2 Cells

Human monocytes

Catalog code: thp-nullz

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

For research use only

Version 20J26-MM

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10^6 THP1-Null2 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 at -20 °C.*

*The expiry date is specified on the product label.

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

- Cells will undergo genotypic changes over time that will result in reduced responsiveness 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-Null2 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), every other passage.

Quality control

- The functionality of THP1-Null2 cells was tested using inflammasome inducers; including Nigericin, Alum Hydroxide, Poly(dA:dT), and *E. coli* OMVs.
- 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. 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-Null2 cells are derived from THP-1 human monocytic cells, the most commonly used model cell line for the study of inflammasome activation. Indeed, they express high levels of NLRP3, ASC and pro-caspase-1[1]. These cells produce IL-1β upon stimulation with canonical or non-canonical inflammasome inducers. The secretion of hIL-1β in the culture supernatant can be assessed using InvivoGen's HEK-Blue™ IL-1β sensor cells. THP1-Null2 cells are the positive control cell line for InvivoGen's THP1-KO-ASC, THP1-KO-NLRP3, and THP1-KO-CASP4. THP1-Null2 cells are resistant to Zeocin™.

1. Schmid-Burgk, J.L *et al.*, 2015. Caspase-4 mediates non-canonical activation of the NLRP3 inflammasome in human myeloid cells. Eur. J. Immunol. 45:2911.

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 activation of PRR signaling pathways.

- **Freezing Medium:** 95 % fetal bovine serum (FBS), 5 % 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:** 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 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). **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.

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 3-622-34-80
E-mail: info@invivogen.com

Cell maintenance

1. THP1-Null2 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-Null2 cells is ~48 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-Null2 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.

EXPERIMENTAL PROCEDURES

THP1-Null2 cells are suited for the study of inflammasome activation. The model of functional inflammasome formation is a two-step process of priming (step 1) followed by activation (step 2). Priming (i.e. with LPS) induces the production of pro-IL-1β, the immature form of IL-1β. Subsequent stimulation with inflammasome inducers, such as Nigericin or Alum Hydroxide, 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 (e.g. using InvivoGen's HEK-Blue™ IL-1β sensor cells).

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

Find below a protocol for assessing the inflammasome response in THP1 Null2 monocytes or macrophages.
If you would like to work with THP1-Null2 macrophages, we recommend that you use the PMA-based differentiation protocol.

PMA-based monocyte differentiation into macrophages (optional)

1. Dispense 20 µl of PMA at 200-500 ng/ml (final concentration: 20-50 ng/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.
4. Carefully remove culture supernatant . Add 200 µl of test medium.
5. Culture for 4 to 7 days at 37 °C in 5% CO_2.

Inflammasome assay

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-Null2 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 THP1-Null2 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
Alum Hydroxide	Inflammasome inducer	tlrl-aloh
HEK-Blue™ IL-1β	IL-1β reporter cells	hkb-il1b
Zeocin™	Selective antibiotic	ant-zn-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-KO-NLRP3	NLRP3 knockout THP-1 cells	thp-konlrp3z

For a full list of our inflammasome inducers, please visit https://www.invivogen.com/inflammasome-inducers

For a full list of our inflammasome test cells, please visit https://www.invivogen.com/inflammasome-test-cells