THP1-KO-CASP4 Cells

Caspase-4 knockout human monocytes

Catalog code: thp-kocasp4z

https://www.invivogen.com/thp1-kocasp4

For research use only

Version 20J26-MM

PRODUCT INFORMATION

Contents

- 3-7 x 10^6 THP1-KO-CASP4 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-CASP4 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 *CASP4* 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.

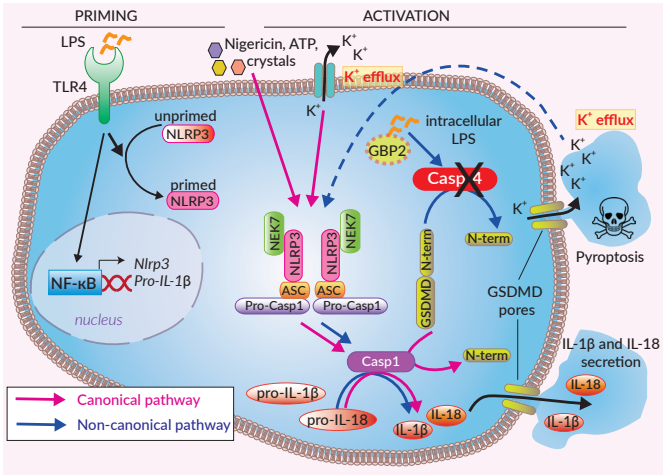
PRODUCT DESCRIPTION

THP1-KO-CASP4 cells were generated from the human monocytic THP1-Null2 cell line through the stable knockout of the caspase-4 (*CASP4*) gene (α and γ isoforms), causing the loss of caspase-4 expression. Although these cells are fully KO for the CASP4 protein, they exhibit some IL-1β secretion after non-canonical inflammasome activation by *E. coli* outer membrane vesicles (OMVs) or transfected LPS. This remnant activity may be attributed to a partial rescue of the non-canonical inflammasome response by CASP5. As expected, the mature IL-1β secretion is not affected upon canonical inflammasome activation.

THP1-KO-CASP4 cells are resistant to Zeocin™.

BACKGROUND

Human caspase-4 (CASP4), CASP5, and their murine ortholog CASP11 are inflammatory caspases that play a crucial role in the non-canonical inflammasome response[1,2]. Independent of TLR4, CASP4 (as well as CASP5 and CASP11) senses intracellular lipopolysaccharide (LPS) that has been released from lysed Gram-negative bacteria as "free" LPS aggregates or actively delivered to the host cell through the release of outer membrane vesicles (OMVs). CASP4 directly binds to the lipid A moiety of LPS, which features a variable number of acylated fatty acid chains depending on the bacterial strain[1,2]. Interestingly, CASP4 has a broader reactivity than its murine ortholog as it can bind hexa-acylated (6 chains) as well as under-acylated (4-5 chains) lipid A[3]. Additionally, an IFN-inducible guanylate-binding protein GBP2 is an important co-factor required to trigger CASP4 activation in response to under-acylated LPS only[3]. As CASP4/5/11 cannot cleave pro-IL-1β/-18, they trigger the cleavage of the pore-forming protein gasdermin D (GSDMD), leading to the release of alarmins and K^+ efflux. Ultimately, this induces the activation of NLRP3 and CASP1-mediated IL-1β/-18 maturation and secretion[1,2].



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. **2. Baker P.J. *et al.*, 2015**. NLRP3 inflammasome activation downstream of cytoplasmic LPS recognition by both caspase-4 and caspase-5. Eur. J. Immunol. 45:2918. **3. Lagrange B. *et al.*, 2018**. Human caspase-4 detects tetra-acylated LPS and cytosolic Francisella and functions differently from murine caspase-11. Nat. Commun. 9:242.

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
- **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 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.

Cell Maintenance

1. THP1-KO-CASP4 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-CASP4 cells is ~44 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-CASP4 cells with less than 20 passages.

EXPERIMENTAL PROCEDURES

THP1-KO-CASP4 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 at 150 x g (RCF) for 10 mins or 300 x g (RCF) for 5 mins.
3. Remove supernatant and resuspend THP1-KO-CASP4 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. 1:2 dilution series of E. coli OMVs starting at 100 µg/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-KO-CASP4 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
Alum Hydroxide	Inflammasome inducer	tlrl-aloh
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

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