THP1-NLRC4 Cells

NLRC4 inflammasome test cell line

Catalog code: thp-nlrc4

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

For research use only

Version 19G02-MM

PRODUCT INFORMATION

Contents and Storage

- 1 vial of THP1-NLRC4 cells (3-7 x 10^6 cells)
- 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 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 to ensure the best cell viability and assay performance. If you are unable to thaw the cells immediately, frozen cells may be placed in liquid nitrogen until you are ready to thaw and propagate them, however, this may reduce cell viability.

Quality control

- Expression of NLRC4 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 confirmed.
- These cells are guaranteed mycoplasma-free.

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-NLRC4 cells should not be passaged more than 20 times to remain fully efficient. THP1-NLRC4 cells should be maintained in growth medium supplemented with blasticidin.

USE RESTRICTIONS

These cells are distributed for research purposes only.

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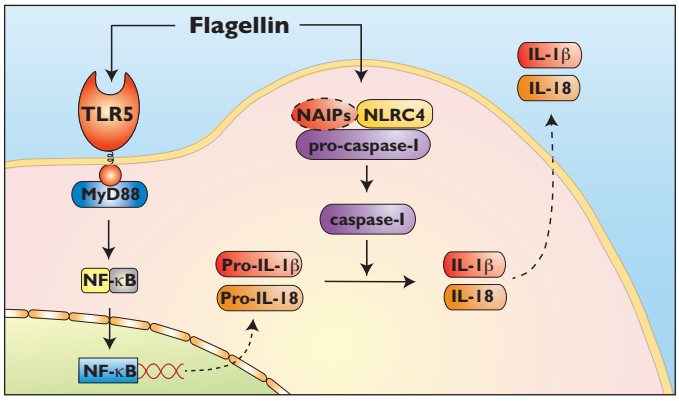
BACKGROUND

Toll-like receptor 5 (TLR5), a surface localized receptor, detects extracellular flagellin resulting in MyD88-mediated NF-κB activation and the production of proinflammatory cytokines, including pro-IL-1β and IL-18. Flagellin can also be injected into the cytosol of mammalian cells by bacteria possessing a type III or type IV secretion systems, such as *Salmonella typhimurium* and *Pseudomonas aeruginosa*. The NOD-like receptor (NLR) protein NLRC4 recognizes cytosolic flagellin triggering the formation of inflammasomes, which leads to the activation of caspase-1 and the subsequent maturation and secretion of IL-1β and IL-18[1, 2]. NAIPs, another subfamily of NLRs, act as adaptors of NLRC4, functioning as receptors for flagellins from certain bacteria.

1. Zhao Y. *et al.*, 2011. The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. Nature. 477:596-600. 2. Kofoed E. & Vance R., 2011. Innate immune recognition of bacterial ligands by NAIPs determines inflammasome specificity. Nature. 477: 592-5.



PRODUCT DESCRIPTION

THP1-NLRC4 cells are derived from the THP1 human monocytic cell line, which represents the most commonly used model cell line to study inflammasome activation. THP1-NLRC4 cells stably overexpress NLRC4 and naturally express TLR5. Stimulation of these cells with flagellin triggers TLR5 signaling leading to NF-κB activation and the production of pro-IL1β. Once in the cytosol, flagellin induces the formation of the NLRC4 inflammasome resulting in the activation of caspase-1 and the release of IL-1β. Levels of IL-1β secreted into the supernatant of THP-1 cells can be monitored using the HEK-Blue™ KD-TLR5 cell line. THP1-NLRC4 and HEK-Blue™ KD-TLR5 cell lines can be used sequentially or co-cultured to save time.

THP1-NLRC4 cells are resistant to blasticidin.

TECHNICAL SUPPORT
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FAQ Any questions about our cell lines ?
Visit our frequently asked questions page

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:**

Option 1 Sequential culture of THP1-NLRC4 & HEK-Blue™ KD-TLR5 cells: RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated fetal bovine serum, Pen-Strep (100 U/ml-100 µg/ml)

Option 2 Co-culture of THP1-NLCR4 & HEK-Blue™ KD-TLR5 cells: IMDM (Iscove's Modified Dulbecco's Media), 10% heat-inactivated fetal bovine serum, Pen-Strep (100 U/ml-100 µg/ml)

Required Selective Antibiotic(s)

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.
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 selection 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. Prepare 1 ml aliquots of cells in 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 10 µg/ml of Blasticidin 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 Recommandations

To ensure the best results:

- ***Use THP1-NLRC4 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-NLRC4 cells are designed to study the activation of the NLRC4 inflammasome. Mature IL-1β can be detected by Western blot, ELISA, or a cell-based assay.

InvivoGen has developed HEK-Blue™ KD-TLR5 cells, a reporter cell line designed to monitor bioactive IL-1β secreted by THP-1 cells upon flagellin-induced NLRC4 activation. HEK-Blue™ KD-TLR5 cells are derived from the HEK293 cell line, which endogenously expresses TLR5 and the IL-1β receptor (IL-1R). This cell line features an NF-κB-inducible SEAP reporter gene and was engineered to knock-down the expression of TLR5 to avoid activation of NF-κB upon flagellin-induced TLR5 stimulation. The knockdown of TLR5 permits the analysis of flagellin specifically for its NLRC4 stimulating activity. Binding of IL-1β to IL-1R initiates a signaling cascade leading to the activation of NF-κB and the subsequent production of SEAP. Detection of SEAP in the supernatant of HEK-Blue™ KD-TLR5 cells can be readily assessed using the QUANTI-Blue™ assay. Levels of IL-1β secreted into the supernatant of THP-1 cells can be monitored using the HEK-Blue™ KD-TLR5 cell line. THP1-NLRC4 and HEK-Blue™ KD-TLR5 cell lines can be used sequentially (option 1) or co-cultured to save time (option 2).

DETECTION OF IL-1β IN THP-1 SUPERNATANTS

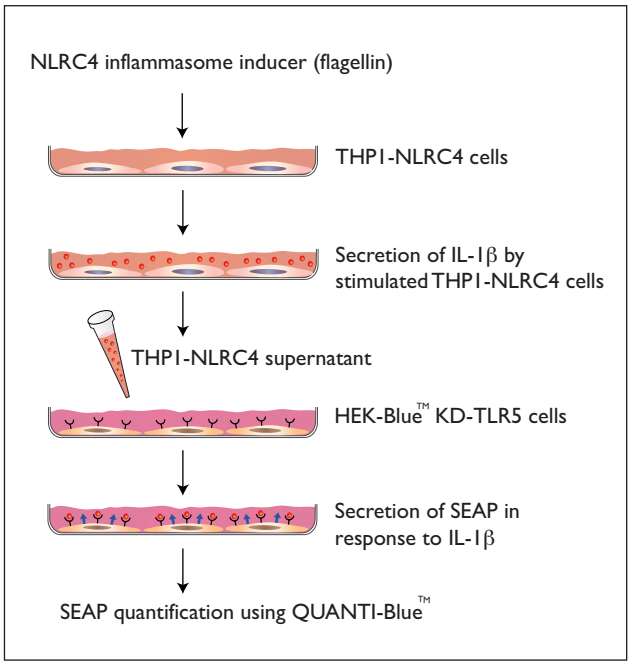

Figure 1. THP-1/HEK-Blue™ IL-1β Assay

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• Option 1: Sequential culture of THP1-NLRC4 & HEK-Blue™ KD-TLR5 cells

Activation of THP1-NLRC4 cells

THP1-NLRC4 cells are grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 µg/ml Normocin™, and Pen-Strep (100 U/ml-100 µg/ml). THP1-NLRC4 cells are grown in suspension to a density of 1 x 10^6 cells/ml in tissue culture flasks.

Day 1

1. Add 20 µl of each sample per well of a flat-bottom 96-well plate.
2. Add 20 µl of a positive control (such as FLA-BS Ultrapure, 200 ng/ml) in one well.
3. Prepare a THP1-NLRC4 cell suspension at 1 x 10^6 cells/ml and add 180 µl of this cell suspension per well of a 96-well plate (2 x 10^5 cells/well).
4. Incubate overnight at 37°C in 5% CO_2.

Detection of IL-1β by HEK-Blue KD-TLR5 cells

HEK-Blue™ KD-TLR5 cells are grown in DMEM supplemented with 10% heat inactivated fetal bovine serum, 2 mM L-glutamine, 100 µg/ml Normocin™, and Pen-Strep (100 U/ml-100 µg/ml).

Day 2

1. Prepare HEK-Blue™ KD-TLR5 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™ KD-TLR5 cells can be altered by the action of trypsin. Do not use trypsin to detach HEK-Blue™ KD-TLR5 cells.
2. Add 50 µl of activated THP1-NLRC4 cell supernatant per 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 growth medium, as a negative control.
4. Add 150 µl of HEK-Blue™ KD-TLR5 cell suspension (~ 5 x 10^4 cells) per well.
5. Incubate overnight at 37°C in 5% CO_2.

Day 3

6. Prepare QUANTI-Blue™ Solution following the instructions on the product data sheet.
7. Add 180 µl of QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
8. Add 20 µl of induced HEK-Blue™ KD-TLR5 cells supernatant.
9. Incubate the plate at 37°C for 1-6 hours.
10. Determine SEAP levels using a spectrophotometer at 620-655 nm.

• Option 2: Co-culture of THP1-NLCR4 & HEK-Blue™ KD-TLR5 cells

Activation of THP1-NLRC4 cells with concurrent detection of IL-1β by HEK-Blue KD-TLR5 cells

Day 1

1. Add 20 µl of each sample per well of a flat-bottom 96-well plate.
2. Add 20 µl of a positive control (such as FLA-BS Ultrapure, 200 ng/ml) in one well.
3. Prepare a THP1-NLRC4 cell suspension in IMDM, 10% heat-inactivated FBS at 2 x 10^6 cells/ml and add 90 µl of this cell suspension, per well of a 96-well plate (2 x 10^5 cells/well).
4. Prepare a HEK-Blue™ KD-TLR5 cell suspension in IMDM, 10% heat-inactivated FBS at 5 x 10^5 cells/ml and add 90 µl of this cell suspension per well of a 96-well plate (5 x 10^4 cells/well).
5. Incubate overnight at 37°C in 5% CO_2.

Day 2

6. Prepare QUANTI-Blue™ Solution following the instructions on the data sheet.
7. Add 180 µl of QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
8. Add 20 µl of cell 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	Cat.
Blasticidin	Selective antibiotic	ant-bl-1
Normocin™	Antimicrobial agent	ant-nr-1
HEK-Blue™ KD-TLR5 cells	TLR5 deficient reporter cells	hkb-kdtlr5
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs
NLRC4 Inflammasome inducer		
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