THP1-Null Cells

Human monocytes
Catalog # thp-null
http://www.invivogen.com/thp1-null

For research use only
Version # 17F28-MM

PRODUCT INFORMATION

Contents and Storage
• 1 vial of THP1-Null cells (3-7 x 10⁶ cells) in freezing medium

IMPORTANT: Cells are shipped frozen. If cells are not frozen upon arrival, contact InvivoGen immediately.

• 1 ml Normocin™ (50 mg/ml). Normocin™ is a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20 °C.*

• 100 µl Hygromycin B Gold (100 mg/ml). Store Hygromycin B Gold at 4°C or 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
• The functionality of THP1-Null cells was tested using inflammasome inducers, such as MSU crystals and ATP.

• The stability of this cell line for 20 passages following thawing has been verified.

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

USE RESTRICTIONS

These cells are distributed for research purposes only.
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PRODUCT DESCRIPTION

THP1-Null cells are derived from THP-1 human monocytic cells, that represent 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-Null cells produce IL-1β upon stimulation with inflammasome inducers, such as MSU crystals and ATP.

As THP1-Null cells are fully efficient for NLRP3 and ASC activities, they are the positive control cell line for InvivoGen’s THP1-defASC and THP1-defNLRP3, that are deficient in ASC and NLRP3 respectively.

THP1-Blue™-Null cells are resistant to hygromycin B.

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)

Notes:
- 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.
- Heat-inactivated FBS is also commercially available.

• Freezing Medium: 90% fetal bovine serum (FBS), 10% DMSO

Required Selective Antibiotic(s)
Hygromycin B Gold (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 1000-1500 RPM (RCF 200-300 g) for 5 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium. **Do not add selective antibiotics.**
6. Transfer the vial contents to a 25 cm² tissue culture flask containing 5 ml of growth medium.
7. Place the culture at 37 °C in 5% CO₂.

Frozen Stock Preparation
1. Resuspend cells at a density of 5-7 x 10⁶ cells/ml in freshly prepared 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 3-4 days by inoculating 7 x 10⁵ cells/ml. Do not allow the cell concentration to exceed 2 x 10⁶ cells/ml.

Cell Handling Recommendations
To ensure the best results:
- **Use THP1-Null 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% CO₂.

APPLICATION
THP1-Null cells are designed for the study of inflammasome activation as they express high levels of NLRP3, ASC and pro-caspase-1. To become susceptible to inflammasome inducers, these 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 alum 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. 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™, a SEAP detection medium. QUANTI-Blue™ turns blue in the presence of SEAP which can be easily quantified using a spectrophotometer.

DETECTION OF IL-1β IN THP-1 SUPERNATANTS

Figure 1. THP-1/HEK-Blue™ IL-1β Assay
Activation of THP1-Null cells
THP1-Null cells are grown in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum, 2 mM L-glutamine and antibacterial antibiotics such as penicillin/streptomycin or Normocin™. THP1-Null cells are grown in suspension to a density of 1.0x10^6 cells/ml in tissue culture flasks.

* Option 1: PMA induction
Day 1
1. Add 180 μl of THP1-Null cell suspension per well of a 96-well plate (~ 1.8 x 10^5 cells/well).
2. Treat THP1-Null cells with 20 μl of PMA at 3 μg/ml or 5 μM (final concentration 300 ng/ml or 0.5 μM) for 3 hours at 37°C in 5% CO2.
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 alum (see Related Products).
6. Incubate overnight at 37°C in 5% CO2.

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

* Option 2: LPS induction
1. Prepare a THP1-Null cell suspension at 2.0 x 10^6 cells/ml and add 180 μl of this cell suspension per well of a 96-well plate (~ 360 x 10^3 cells/well).
2. Treat THP1-Null cells with 20 μl of LPS at 10 μg/ml for 3 hours at 37°C in 5% CO2.
3. Gently remove medium and add 180 μl of supplemented RPMI.
4. Add 20 μl of an inflammasome inducer, such as ATP or alum.
5. Incubate overnight at 37°C in 5% CO2.

Detection of IL-1β by HEK-Blue IL-1β cells
Day 1
1. Prepare 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 ~330,000 cells/ml.

Note: To ensure the best results, use a culture maintained around 6.10^5 cells/ml or 70 to 80% confluence.
- 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-Null 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 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% CO2.

Day 2
6. Prepare QUANTI-Blue™ following the instructions on the enclosed product data sheet.
7. Add 150 μl of resuspended QUANTI-Blue™ per well of a flat-bottom 96-well plate.
8. Add 50 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

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