THP1-HMGB1-Lucia™ Cells
Pyroptosis and necroptosis reporter monocytes
Catalog code: thp-gb1lc
https://www.invivogen.com/thp1-hmgb1-lucia
For research use only
Version 21G22-MM

PRODUCT INFORMATION
Contents
• 3-7 x 10^6 THP1-HMGB1-Lucia™ Cells in a flask shipping or cryovial.

IMPORTANT: If cells provided in a cryovial are not frozen upon arrival, contact InvivoGen immediately.
• 1 ml of Normocin™ (50 mg/ml), a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20 °C.
• 100 μl of Zeocin™ (100 mg/ml). Store at 4 °C or at -20 °C.
• 1 pouch of QUANTI-Luc™ (Lucia luciferase detection medium) Store QUANTI-Luc™ pouch at -20 °C for 12 months. Reconstituted QUANTI-Luc™ medium is stable for 1 week at 4 °C and for 1 month at -20 °C. Protect QUANTI-Luc™ from light.

Note: Data sheets for all components are available on our website.
*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.
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. Cells will undergo genotypic changes resulting in reduced responsiveness over time in normal cell culture conditions. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages. THP1-HMGB1-Lucia™ cells should not be passaged more than 20 times to remain fully efficient. THP1-HMGB1-Lucia™ cells should be maintained in growth medium supplemented with the selective antibiotic Zeocin™ (100 μg/ml) following every other passage.

Quality control
• The functionality of THP1-HMGB1-Lucia™ cells was tested using inflammasome inducers, such as nigericin.
• The stability of this cell line for 20 passages following thawing has been verified.
• THP1-HMGB1-Lucia 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 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.

BACKGROUND
Pyroptosis and necroptosis are two forms of necrotic programmed cell death characterized by the release of the alarmin HMGB1 (High Mobility Group Box-1) upon cell membrane rupture.

Pyroptosis
Pyroptosis is a consequence of inflammasome activation. Inflammasomes are complexes consisting of a cytoplasmic sensor (AIM2, Pyrin, NLRP1, NLRP3 or NLRC4), an adaptor (ASC: apoptosis-associated speck-like protein), and pro-caspase-1. Typically, inflammasome activation is a two-step process. A first signal (priming), provided by microbial molecules such as lipopolysaccharide (LPS), induces NF-κB-dependent expression of pro-IL1β. The second signal, provided by structurally unrelated microbial molecules (e.g. nigericin toxin) or danger signals, triggers inflammasome multimerization. This leads to caspase-1 self-activation, proteolytic maturation of IL-1β and IL-18, and cleavage of Gasdermin D (GSDMD). Subsequent GSDMD pore formation at the cell membrane elicits a rapid cell death associated with the release of IL-1β, IL-18 and HMGB1.

Necroptosis
Necroptosis may be induced upon Tumor Necrosis Factor (TNF) receptor or pattern recognition receptors (PRRs) activation. In the case of TNF-α induced necroptosis, RIPK1 (receptor-interacting serine/threonine-protein kinase 1) is recruited to the stimulated TNF receptor along with TRADD (TNF-associated death domain), FADD (Fas-associated protein with death domain), and cIAP (cellular inhibitor of apoptosis). When cIAP and caspase-8 are absent or inactive, RIPK1 associates with RIPK3 and MLKL (mixed lineage kinase-like) as a necosome. Then phosphorylated MLKL relocates at the plasma membrane where it drives an influx of ions, most probably by pore formation, leading to cell lysis and release of HMGB1.

**PRODUCT DESCRIPTION**

THP-1-HMGB1-Lucia™ cells are pyroptosis and necroptosis reporter cells. They derive from THP-1 human monocytic cell line, widely used to study regulated necrosis. THP-1-HMGB1-Lucia™ cells stably express in the cytoplasm a 46.5 kDa fusion protein, HMGB1::Lucia, in which the C-terminus of HMGB1 is fused to the Lucia luciferase. These cells respond to commonly used inflammasome inductors such as Nigericin, and to necroptosis cocktail inducers such as TNF receptor or PRR agonist + Z-VAD-FMK (pan-caspase inhibitor) + BV6 (cIAP inhibitor). Following necroptosis or inflammasome-mediated-pyroptosis, pores are formed in the cell membrane and HMGB1::Lucia is released in the extracellular milieu. Levels of HMGB1::Lucia in the supernatant can be readily monitored by measuring the light signal produced after addition of QUANTI-Luc™.

Note: Inflammasome induction also triggers IL-1β and IL-18 secretion by THP-1-HMGB1-Lucia™ cells (see Box 1 on the last page).

THP-1-HMGB1-Lucia™ cells are resistant to Zeocin™.

**HANDLING PROCEDURES**

**Required Cell Culture Medium**

- **Growth Medium:** RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated fetal bovine serum (FBS; 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 activation of the reporter gene.

- **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)

- **Freezing Medium:** 95% FBS, 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 must 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 into a larger vial containing 15 ml of pre-warmed growth medium (with 20% heat-inactivated FBS). Do not add selective antibiotics until the cells have been passaged twice.
4. Centrifuge vial at 150 x g (RCF) for 10 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium (with 20% heat-inactivated FBS).
6. Transfer the vial contents to a 25 cm² tissue culture flask containing 5 ml of growth medium (with 20% heat-inactivated FBS).
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.

**Cell maintenance**

- After cells have recovered (after at least two passages), subculture the cells in growth medium (with 10% heat-inactivated FBS). To maintain selection pressure, add 100 μg/ml of Zeocin™ to the growth medium every other passage.
- Pass the cells every 3 days by inoculating 5 x 10⁶ cells/ml. Do not allow the cell concentration to exceed 2 x 10⁶ cells/ml.

**Cell Handling Recommendations**

To ensure the best results:
- Use THP-1-HMGB1-Lucia™ 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.

**SAFETY CONSIDERATIONS**

**Biosafety Level 1**

**EXPERIMENTAL PROCEDURES**

**Assay Principle and Features**

This assay is an alternative to the lactate dehydrogenase (LDH) assay which measures the activity of LDH released upon rupture of cell membrane integrity. The THP-1-HMGB1-Lucia™ cellular assay relies on the luminescence quantification of the HMGB1::Lucia fusion protein released in the supernatant upon pyroptosis or necroptosis.

**Pyroptosis assay**

It is recommended to perform the assay with test medium which does not contain Normocin™ nor Zeocin™.

1. Add 20 μl of LPS-EK (1 μg/ml final concentration) per well of a flat-bottom 96-well plate.
2. Prepare a THP-1-HMGB1-Lucia™ cell suspension at ~1 x 10⁶ cells/ml.
3. Dispense 180 μl of cell suspension (~200,000 cells) per well.
4. Incubate at 37 °C in 5% CO₂ for 3 h.
5. Gently remove medium and add 180 μl of fresh test medium.
6. Add 20 μl of an inflammasome inducer, such as Nigericin (12.5 μM final concentration).
7. Incubate at 37 °C in 5% CO₂ for 16-24 h. Proceed to detection of HMGB1::Lucia using QUANTI-Luc™ as described on the next page.

**Detection of HMGB1::Lucia**

- 1st Signal: e.g. PMA or LPS
- 2nd Signal: e.g. Nigericin
- Measurement: Luminescence

**Handout:** QUANTI-Luc™ PLUS HMGB1::Lucia

**Visit our FAQ page.**

Any questions about our cell lines?

**TECHNICAL SUPPORT**

InvivoGen USA (Toll-Free): 888-457-5873
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InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com
Necroptosis assay
It is recommended to perform the assay with test medium which does not contain Normocin™ nor Zeocin™.
1. Add 20 μl of a caspase inhibitor such as Z-VAD-FMK (25 μM final concentration) per well of a flat-bottom 96-well plate.
2. Prepare a THP1-HMGB1-Lucia™ cell suspension at ~1 x 10^6 cells/ml.
3. Dispense 180 μl of cell suspension (~200,000 cells) per well.
4. Incubate at 37°C in 5% CO_2 for 1 h.
5. Add 20 μl of a cIAP inhibitor such as BV6 (5 μM final concentration) and TNF-α (100 ng/ml final concentration) per well.
6. Incubate the plate at 37°C in a CO_2 incubator for 8-24 h. Proceed to detection of HMGB1::Lucia using QUANTI-Luc™ as described below.

Detection of HMGB1::Lucia
Below is a protocol for end-point readings using a luminometer. This protocol can be adapted for use with kinetic measurements.
1. Prepare the QUANTI-Luc™ assay solution following the instructions on the enclosed data sheet.
2. Transfer 10 μl of THP1-HMGB1-Lucia™ stimulated cell supernatant into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Add 50 μl of QUANTI-Luc™
4. Proceed immediately with the measurement.

Box 1
Inflammasome activation triggers the extracellular release of mature IL-1β and IL-18. Levels of IL-1β can be measured by Western blot, ELISA or using InvivoGen’s HEK-Blue™ IL-1β cellular assay. Alternatively, secreted IL-1β can be detected using InvivoGen’s HEK-Blue™ IL-18 cellular assay.

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<tr>
<th>Product</th>
<th>Description</th>
<th>Cat. Code</th>
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<tr>
<td>HEK-Blue™ IL-1β</td>
<td>IL-1β reporter cells</td>
<td>hkb-il1b</td>
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<tr>
<td>HEK-Blue™ IL-18</td>
<td>IL-18 reporter cells</td>
<td>hkb-hml18</td>
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<td>LPS-EK (E. coli K12)</td>
<td>TLR2/TLR4 agonist</td>
<td>ttrl-eklps</td>
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<td>MSU Crystals</td>
<td>Inflammasome inducer</td>
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<td>Nigericin</td>
<td>Inflammasome inducer</td>
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<td>Normocin™</td>
<td>Antimicrobial agent</td>
<td>ant-nr-1</td>
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<td>Lucina detection medium</td>
<td>rep-qlc1</td>
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<td>Zeocin™</td>
<td>Selection antibiotic</td>
<td>ant-zn-1</td>
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<td>Z-VAD-FMK</td>
<td>Caspase inhibitor</td>
<td>ttrl-vad</td>
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For a full list of the inflammasome inducers provided by InvivoGen, visit [https://www.invivogen.com/inflammasome-inducers](https://www.invivogen.com/inflammasome-inducers).
QUANTI-Luc™
A coelenterazine-based luminescence assay reagent
Catalog code: rep-qlc1, rep-qlc2
https://www.invivogen.com/quanti-luc

For research use only
Version 19A04-MM

PRODUCT INFORMATION
Contents
QUANTI-Luc™ is provided as packs of individually sealed pouches.
• rep-qlc1: 2 pouches of QUANTI-Luc™
• rep-qlc2: 5 pouches of QUANTI-Luc™
Each pouch contains everything needed to prepare 25 ml of reagent allowing the preparation of 500 wells of a 96-well plate.

Storage and Stability
- Store QUANTI-Luc™ pouches at -20°C for 12 months.
- Reconstituted QUANTI-Luc™ is stable for 1 week at 4°C and for 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.
Note: This product is photosensitive and should be protected from light.

DESCRIPTION
QUANTI-Luc™ is an assay reagent containing all the components required to quantitively measure the activity of Lucia luciferase and other coelenterazine-utilizing luciferases. QUANTI-Luc™ contains the coelenterazine substrate and stabilizing agents for the luciferase reaction. The light signal produced is quantified using a luminometer and expressed as relative light units (RLU). The signal produced correlates to the amount of luciferase protein expressed, indicating promoter activity in the reporter assay.

QUANTI-Luc™ is optimized for use with Lucia luciferase reporter cell lines. Lucia luciferase is a secreted coelenterazine luciferase encoded by a synthetic gene. As Lucia luciferase is secreted, it can be directly measured in the cell culture medium using bioluminescent assays.

InvivoGen provides a recombinant Lucia luciferase protein (see Related Products) which is a positive control for QUANTI-Luc™. A dilution series of the recombinant Lucia luciferase protein can also be used to determine the linear range of the assay.

METHODS
Preparation of QUANTI-Luc™
1. Pour the pouch contents into a 50 ml screw cap tube.
2. Add 25 ml of sterile water.
3. Swirl product gently until powder is completely dissolved.
4. Use QUANTI-Luc™ assay solution immediately or store until required for use. Reconstituted QUANTI-Luc™ can be stored for 1 week at 4°C and for 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.
Note: This product is photosensitive and should be protected from light.

Detection of luciferase activity from cell culture medium
To obtain end-point readings using a luminometer with an injector.
1. Set the luminometer with the following parameters: 50 µl of injection, end-point measurement with a 4 second start time and 0.1 second reading time.
2. Pipet 10-20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Prime the injector with the QUANTI-Luc™ assay solution and proceed immediately with the measurement.

To obtain end-point readings using a luminometer without injectors.
1. Set the luminometer with a 0.1 second reading time.
2. Pipet 10-20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Add 50 µl of QUANTI-Luc™ assay solution to each well or tube.
4. Gently tap the plate several times to mix (do not vortex).
5. Proceed immediately with the measurement.

RELATED PRODUCTS

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<tr>
<th>Product</th>
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</thead>
<tbody>
<tr>
<td>QUANTI-Luc™ Gold (For standard and HTS assays)</td>
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<tr>
<td>pSelect-zeo-Lucia™ (expression plasmid)</td>
<td>psetz-lucia</td>
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<tr>
<td>Recombinant Lucia luciferase protein</td>
<td>rec-lucia</td>
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<td>Reporter Cells</td>
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<tr>
<td>THP1-Dual™ (IRF-Lucia/NF-κB-SEAP) Cells</td>
<td>thpd-nfis</td>
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<tr>
<td>THP1-Lucia™ NF-κB Cells</td>
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For a complete list of InvivoGen's Lucia luciferase Reporter Cells visit https://www.invivogen.com/lucia-reporter-cells.