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 23A09-MM

PRODUCT INFORMATION

Contents

- **3-7 x 10^6 of 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 3 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.*

*The expiry date is specified on the product label.

Note: Data sheets for all components are available on our website.

- **1 tube of QUANTI-Luc™ 4 Reagent**, a Lucia luciferase detection reagent (sufficient to prepare 25 ml). Store at -20 °C. Avoid repeated freeze-thaw cycles. *Note: This product is photosensitive and should be protected from light.*

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

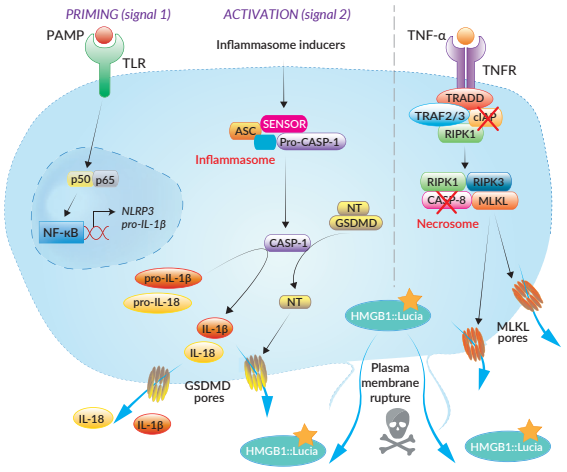
Pyroptosis and necroptosis are two forms of necrotic programmed cell death characterized by the release of the alarmin HMGB1 (High Mobility Group Box-1) upon cell membrane rupture.

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[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[1,2].

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 necrosome. Then phosphorylated MLKL relocalizes at the plasma membrane where it drives an influx of ions, most probably by pore formation, leading to cell lysis and release of HMGB1[3].



1. Broz P. and Dixit V.M., 2016. Inflammasomes: mechanism of assembly, regulation and signalling. Nat Rev Immunol. 16:407-20. 2. Kovacs S.B. and Miao E.A., 2017. Gasdermins: effectors of pyroptosis. Trends Cell Biol. 27:673-84. 3.Grootjans S. *et al.*, 2017. Initiation and execution mechanisms of necroptosis: an overview. Cell Death Differ. 24:1184-95.

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FAQ Any questions about our cell lines? Visit our FAQ page.

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PRODUCT DESCRIPTION

THP1-HMGB1-Lucia™ cells are pyroptosis and necroptosis reporter cells. They derive from THP-1 human monocytic cell line, widely used to study regulated necrosis. THP1-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 inducers 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™ 4 Lucia/Gaussia.

Note: Inflammasome induction also triggers IL-1β and IL-18 secretion by THP1-HMGB1-Lucia™ cells (see Box 1 on the last page).

THP1-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 cm^2 tissue culture flask containing 5 ml of growth medium (with 20% heat-inactivated FBS).
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 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 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 10^5 cells/ml. Do not allow the cell concentration to exceed 2 x 10^6 cells/ml.

Cell Handling Recommendations

To ensure the best results:

- ***Use THP1-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% CO_2.*

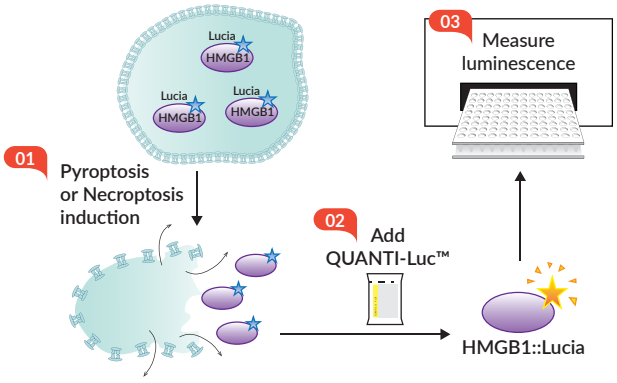
SAFETY CONSIDERATIONS

Biosafety Level 1

EXPERIMENTAL PROCEDURES

Assay Principle and Features

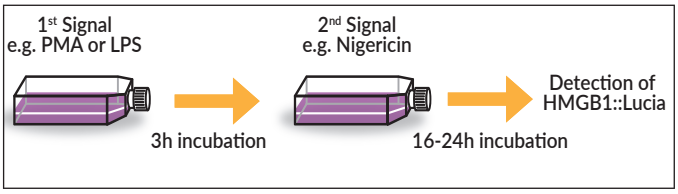
This assay is an alternative to the lactate deshydrogenase (LDH) assay which measures the activity of LDH released upon rupture of cell membrane integrity. The THP1-HMGB1-Lucia™ cellular assay relies on the luminescence quantification of the HMGB1::Lucia fusion protein released in the supernatant upon 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 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 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% CO_2 for 16-24 h. Proceed to detection of HMGB1::Lucia using QUANTI-Luc™ 4 Lucia/Gaussia as described on the next page.



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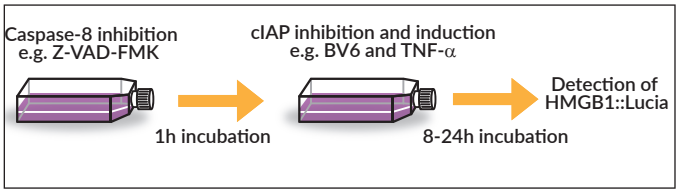
Necroptosis assay

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™ 4 Lucia/Gaussia 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™ 4 Lucia/Gaussia 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 Lucia/Gaussia.
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-18 can be detected using InvivoGen's HEK-Blue™ IL-18 cellular assay.

RELATED PRODUCTS

Product	Description	Cat. Code
HEK-Blue™ IL-1β	IL-1β reporter cells	hkb-il1bv2
HEK-Blue™ IL-18	IL-18 reporter cells	hkb-hmil18
LPS-EK (*E. coli* K12)	TLR2/TLR4 agonist	tlrl-eklps
MSU Crystals	Inflammasome inducer	tlrl-msu
Nigericin	Inflammasome inducer	tlrl-nig
Normocin™	Antimicrobial agent	ant-nr-1
Poly(dA:dT)/LyoVec™	Inflammasome inducer	tlrl-patc
QUANTI-Luc™ 4 Lucia/Gaussia	Luminesence detection kit	rep-qlc4lg1
Zeocin®	Selection antibiotic	ant-zn-1
Z-VAD-FMK	Caspase inhibitor	tlrl-vad

For a full list of the inflammasome inducers provided by InvivoGen, visit https://www.invivogen.com/inflammasome-inducers.

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QUANTI-Luc™ 4 Reagent

A coelenterazine-based luminescence assay reagent

https://www.invivogen.com/quanti-luc

For research use only

Version 23A16-MM

PRODUCT INFORMATION

Contents

- 1 tube of **QUANTI-Luc™ 4 Reagent (20X)**

One tube of QUANTI-Luc™ 4 Reagent is sufficient for 5 x 96-well plates (25 ml standard Flash/end-point detection).

Note: This sample cannot be sold separately from the QUANTI-Luc™ 4 Lucia/Gaussia kit.

QUANTI-Luc™ 4 Lucia/Gaussia comprises two liquid components:

- QUANTI-Luc™ 4 Reagent 20X (coelenterazine substrate)
- QUANTI-Luc™ 4 Stabilizer 25X (optimized Glow assay reagent)

Find more information at https://www.invivogen.com/quanti-luc.

Storage and Stability

– Store QUANTI-Luc™ 4 Reagent at -20°C for up to 12 months.
– After preparation, the working solution is stable for 48 hours at 4°C and 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.
Note: This product is photosensitive and should be protected from light.

Quality Control

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.
– Physicochemical characterization (pH, appearance).
– Functional assays using recombinant Lucia protein or reporter cells.

DESCRIPTION

QUANTI-Luc™ 4 Reagent is a component of the QUANTI-Luc™ 4 Lucia/Gaussia kit. It contains the coelenterazine substrate for the detection of secreted Lucia or Gaussia activity in live-cell supernatants, and of intracellular Renilla after cell lysis. The light signal produced correlates to the amount of luciferase protein expressed. It is quantified using a luminometer and expressed as relative light units (RLUs).

METHODS

Preparation of QUANTI-Luc™ 4 Reagent working solution (1X):

1. Dilute the total volume of the 20X tube (1.25 ml) of Reagent into 23.75 ml of sterile water to obtain 25 ml of working solution.
2. Vortex **very briefly** (a few seconds).
3. Use the working solution immediately or store until required for use. QUANTI-Luc™ 4 Reagent working solution can be stored for 48 hours at 4°C or 1 month at -20°C.

Flash 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 20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Prime the injector with QUANTI-Luc™ 4 Reagent 1X 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 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™ 4 Reagent 1X 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

Product	Cat. Code
QUANTI-Luc™ 4 Lucia/Gaussia Kit	
500 tests	rep-qlc4lg1
2 x 500 tests	rep-qlc4lg2
5 x 500 tests	rep-qlc4lg5

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