THP1-Lucia™ NF-κB Cells

NF-κB Lucia™ Reporter Monocytes

Catalog code: thpl-nfkbv2

https://www.invivogen.com/thp1-lucia-nfkb

For research use only

Version 24B08-AK

PRODUCT INFORMATION

Contents and Storage

- **3-7 x 10^6 of THP1-Lucia™ NF-κB 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 Zeocin®** (100 mg/ml). Store at 4 °C or at -20 °C.*
- **1 ml of Normocin™** (50 mg/ml), a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20 °C.*

*The expiry date is specified on the product label.

- **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 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'.

Quality control

- Reporter activity has been validated using functional assays.
- The stability for 20 passages following thawing has been verified.
- 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.

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 to 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.

PRODUCT DESCRIPTION

THP1-Lucia™ NF-κB cells were specifically designed for monitoring the NF-κB signal transduction pathway in a physiologically relevant cell line. THP1-Lucia™ NF-κB cells were derived from the human THP-1 monocyte cell line by stable integration of an NF-κB-inducible Lucia™ reporter construct. THP1-Lucia™ NF-κB cells feature the Lucia luciferase gene, a secreted luciferase reporter gene, driven by an IFN-β minimal promoter fused to five copies of the NF-κB consensus transcriptional response element and three copies of the c-Rel binding site. As a result, THP1-Lucia™ NF-κB cells allow the monitoring of NF-κB activation by determining the activity of Lucia luciferase. The levels of NF-κB-induced Lucia™ in the cell culture supernatant are readily assessed with QUANTI-Luc™ 4 Lucia/Gaussia, a Lucia and Gaussia luciferase detection reagen.

THP1-Lucia™ NF-κB cells induce the activation of NF-κB in response to various PRR ligands.

THP1-Lucia™ NF-κB cells are resistant to Zeocin®.

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; 30 min at 56°C), 100 U/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml Normocin™

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.

- **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®

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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.

Notes: 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 (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 freezing medium prepared extemporaneously with cold growth 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.

Notes: If properly stored, cells should remain stable for years.

Cell maintenance

1. After cells have recovered (after at least one passage), 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.
2. Pass the cells every 3-4 days by inoculating 7 x 10^5 cells/ml. Do not allow the cell concentration to exceed 2 x 10^6 cells/ml.

Notes: To ensure the best results:

- Use THP1-Lucia™ NF-κB 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.

SAMPLE PREPARATION

1. Resuspend all powdered samples in endotoxin-free water to avoid activation of TLR4 of the THP-1 cell line.
2. Warm the samples at 37°C before use.

Notes:

- Avoid testing of pure samples soluble only in ethanol or DMSO: these solutions are toxic to the cell line and can result in false negative results.

- We recommend to ensure the absence of cytotoxicity of the sample on cells before running TLR activity detection test. If a cytotoxic effect is observed, the samples should be diluted in endotoxin-free water before testing.

REPORTER ASSAY PROTOCOL

Below is a protocol using THP1-Lucia™ NF-κB cells to monitor the NF-κB reponses upon stimulation with PRR ligands. It is recommended to perform the assay with test medium which does not contain Normocin™nor Zeocin.

This protocol is for **end-point readings** using a luminometer with an injector, this protocol can be adapted for use with kinetic measurements or a luminometer with a manual set-up.

Cell preparation

1. Centrifuge cells at 150 x g (RCF) for 10 minutes or at 300 x g (RCF) for 5 minutes.
2. Remove supernatant and resuspend THP1-Lucia™ NF-κB cells at 5 x 10^5 cells/ml in freshly prepared test medium.

Detection of TLR stimulation

1. Add 20 µl of sample per well including a positive control (such as the TLR2 ligand Pam3CSK4) and endotoxin free water as a negative control (use new tips for each well to avoid cross-contamination).
2. Add 180 µl of cell suspension (~100,000 cells) per well of a flat-bottom 96-well plate.
3. Incubate the plate at 37°C in a CO_2 incubator for 18-24 h.
4. Prepare QUANTI-Luc™ 4 Reagent working solution following the instructions on the pouch.
5. 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.
6. Pipet 10 µl THP1-Lucia™ NF-κB cell culture medium per well into a 96-well white (opaque) or black plate, or a luminometer tube.
7. Prime the injector with QUANTI-Luc™ 4 Reagent working solution and proceed with the measurement.

RELATED PRODUCTS

Product	Description	Cat. Code
FLA-ST Ultrapure	TLR5 ligand	tlrl-pstfla
FSL-1	TLR2 ligand	tlrl-fsl
HKLM	TLR2 ligand	tlrl-hklm
LPS-EB Ultrapure	TLR4 ligand	tlrl-3pelps
MDP	NOD2 ligand	tlrl-mdp
Normocin™	Antimicrobial agent	ant-nr-1
Pam3CSK4	TLR2 ligand	tlrl-pms
QUANTI-Luc™ 4 Lucia/Gaussia	Luminesence detection kit	rep-qlc4lg1
R848	TLR7/8 ligand	tlrl-r848
TLR RT-Primer Set	RT-PCR primers	rts-htlrs
Tri-DAP	NOD1 ligand	tlrl-tdap
Zeocin®	Selection antibiotic	ant-zn-1

QUANTI-Luc™ 4 Reagent

A coelenterazine-based luminescence assay reagent

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

For research use only

Version 23F27-AK

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 or Renilla kits.

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 one component of the QUANTI-Luc™ 4 Lucia/Gaussia and QUANTI-Luc™ 4 Renilla kits. 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 Lucia luciferase activity in 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 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 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™ 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 comprising QUANTI-Luc™ 4 Reagent & Stabilizer	rep-qlc4lg1
QUANTI-Luc™ 4 Renilla Kit comprising QUANTI-Luc™ 4 Reagent & Lysis buffer	rep-qlc4r1

TECHNICAL SUPPORT
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