THP1-Lucia™ NF-κB Cells

NF-κB Lucia™ Reporter Monocytes

Catalog # thpl-nfk

http://www.invivogen.com/thp1-lucia-nfk

For research use only

Version # 17F28-MM

PRODUCT INFORMATION

Contents and Storage
• 1 vial of THP1-Lucia™ NF-κB cells (3-7 x 10^6 cells) in freezing medium
• 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 Zeocin™ (100 mg/ml). Store Zeocin™ at 4°C or at -20°C.*

The expiry date is specified on the product label.
• 1 pouch of QUANTI-Luc™. 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. Keep reconstituted QUANTI-Luc™ away from light.
• 10^9 cells HKLM (Heat killed Listeria monocytogenes; positive control of TLR2 activity). Store HKLM at 4°C for 1 year. Store reconstituted HKLM at 4°C for 1 month or at -20°C for 6 months.

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
• TLR expression was determined by RT-PCR in THP1-Luc™ NF-κB cells. All TLR mRNAs were detected. Considering the concentration of ligands used to stimulate these cells, TLR2, TLR1/2 and TLR2/6 responses are considered to be very strong (see figure 1). TLR4, TLR5 and TLR7/8 responses are robust. Responses to TLR3 and TLR9 are hardly detectable even when high concentrations of the cognate ligands are used.
• THP1-Lucia™ NF-κB cells respond to NOD1 and NOD2 agonists.
• The stability of this cell line for 20 passages following thawing has been verified.
• THP1-Lucia™ NF-κB 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 the terms and conditions of all applicable Limited Use Label Licenses.

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

www.invivogen.com

Figure 1: NF-κB response of THP1-Lucia™ NF-κB cells. Cells were incubated with 10^6 cells/ml HKLM (TLR2), 1 ng/ml Pam3CSK4(TLR1/2), 0.1 ng/ml FSL-1 (TLR2/6), 10 µg/ml poly(I:C) (TLR3), 1 µg/ml LPS-EK Ultrapure (TLR4), 100 ng/ml FLA-ST UP (TLR5), 1 µg/ml R848 (TLR7/8), 10 µg/ml ODN2006 (TLR9), 10 µg/ml Tri-DAP (NOD1) or 10 µg/ml MDP (NOD2). After 24h incubation, the levels of NF-κB-induced Lucia were assessed from the cell culture supernatant using QUANTI-Luc™.

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™ gene, a new secreted luciferase reporter gene, driven by an IFN-β minimal promoter fused to two 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 activity by determining the activity of Lucia™. The levels of NF-κB-induced Lucia™ in the cell culture supernatant are readily assessed with QUANTI-Luc™, a Lucia™ detection reagent.

THP1-Lucia™ NF-κB cells induce the activation of NF-κB in response to various PRR ligands, (see figure 1).

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% heat-inactivated fetal bovine serum (30 min at 56°C), 100 μg/ml Normocin™, Pen-Strep (100 U/ml-100 μg/ml)  
  
  **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.  
  - Heat-inactivated FBS is also commercially available.  
  - **Freezing Medium**: 90% fetal bovine serum (FBS), 10% 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 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 10⁵ 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.  
  
  **Note:** 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 10⁵ cells/ml. Do not allow the cell concentration to exceed 2 x 10⁶ cells/ml.  
  
  **Note:** To ensure the best results:  
  - Use THP1-Lucia™ NF-kB 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.  

DETECTION OF TLR STIMULATION  
Positive control preparation (HKLM)  
1. Prepare a 10⁹ cells/ml solution of HKLM by adding 1 ml of sterile water to the content of the tube.  
2. Mix vigorously by vortexing.  

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.  

TLR Assay  
Below is a protocol 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.  
1. Centrifuge cells at 1000-1500 RPM (RCF 200-300 g) for 5 minutes.  
2. Remove supernatant and resuspend THP1-Lucia™ NF-kB cells at 5 x 10⁵ cells/ml in fresh, pre-warmed growth medium.  
3. Add 20 μl of sample per well including HKLM as the positive control and endotoxin free water as a negative control (use new tips for each well to avoid cross-contamination).  
4. Add 180 μl of cell suspension (~100,000 cells) per well of a flat-bottom 96-well plate.  
5. Incubate the plate at 37°C in a CO₂ incubator for 18-24 h.  
6. Prepare QUANTI-Luc™ following the instructions on the pouch.  
7. Set the luminometer with the following parameters: 50 μl of injection, injector, this protocol can be adapted for use with kinetic measurements or a luminometer with a manual set-up.  
6. Prepare QUANTI-Luc™ following the instructions on the pouch.  
7. Set the luminometer with the following parameters: 50 μl of injection, 4 second start time and 0.1 second reading end-point measurement with a 4 second start time and 0.1 second reading time.  
8. Pipet 10 μl THP1-Lucia™ NF-kB cell culture medium per well into a 96-well white (opaque) or black plate, or a luminometer tube.  
9. Prime the injector with the QUANTI-Luc™ assay solution and proceed with the measurement.  

RELATED PRODUCTS  

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Catalog Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLA-ST Ultrapure</td>
<td>TLR5 ligand</td>
<td>tlr-psfla</td>
</tr>
<tr>
<td>FSL-1</td>
<td>TLR2 ligand</td>
<td>tlr-fsl</td>
</tr>
<tr>
<td>HKL1M</td>
<td>TLR2 ligand</td>
<td>tlr-hklm</td>
</tr>
<tr>
<td>LPS-EB Ultrapure</td>
<td>TLR4 ligand</td>
<td>tlr-3pelps</td>
</tr>
<tr>
<td>MDP</td>
<td>NOD2 ligand</td>
<td>tlr-mdp</td>
</tr>
<tr>
<td>Normocin™</td>
<td>Antimicrobial agent</td>
<td>ant-nr-1</td>
</tr>
<tr>
<td>Pam3CSK4</td>
<td>TLR2 ligand</td>
<td>tlr-pms</td>
</tr>
<tr>
<td>QUANTI-Luc™</td>
<td>Luminesence assay reagent</td>
<td>rep-qlc1</td>
</tr>
<tr>
<td>R848</td>
<td>TLR7/8 ligand</td>
<td>tlr-r848</td>
</tr>
<tr>
<td>TLR RT-Primer Set</td>
<td>RT-PCR primers</td>
<td>rts-htrs</td>
</tr>
<tr>
<td>Tri-DAP</td>
<td>NOD1 ligand</td>
<td>tlr-tdap</td>
</tr>
<tr>
<td>Zeocin™</td>
<td>Selective antibiotic</td>
<td>ant-zn-1</td>
</tr>
</tbody>
</table>
QUANTI-Luc™
A coelenterazine-based luminescence assay reagent
Catalog # rep-qlc1, rep-qlc2
http://www.invivogen.com/quanti-luc

For research use only
Version # 17C17-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™ 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™ reporter cell lines (see Related Products). Lucia™ is a new secreted coelenterazine luciferase encoded by a synthetic gene. As Lucia™ is secreted, it can be directly measured in the cell culture medium using bioluminescent assays.

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

METHODS

Preparation of QUANTI-Luc™
Prepare the QUANTI-Luc™ assay solution as follows:
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.

Detection of luciferase activity from cell culture medium
Prepare the QUANTI-Luc™ assay solution as described above.

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 the following parameters: 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. 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

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>pNiFty3-Lucia™ (reporter plasmid)</td>
<td>pnf3-lc1</td>
</tr>
<tr>
<td>pSelect-zeo-Lucia™ (expression plasmid)</td>
<td>psetz-lucia</td>
</tr>
<tr>
<td>RAW-Lucia™ ISG cells</td>
<td>rawl-isg</td>
</tr>
<tr>
<td>Recombinant Lucia™ protein</td>
<td>rec-lucia</td>
</tr>
<tr>
<td>THP1-Lucia™ NF-kB cells</td>
<td>thpl-nfkb</td>
</tr>
</tbody>
</table>

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com