THP1-Blue™ NF-κB Cells

NF-κB SEAP Reporter Monocytes

Catalog # thp-nfkb

For research use only

Version # 15C23-MM

PRODUCT DESCRIPTION

THP1-Blue™ NF-κB cells were specifically designed for monitoring the NF-κB signal transduction pathway in a physiologically relevant cell line. THP1-Blue™ were derived from the human THP-1 monocyte cell line by stable integration of an NF-κB-inducible SEAP reporter construct. THP1-Blue™ NF-κB cells express a secreted embryonic alkaline phosphatase (SEAP) 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-Blue™ NF-κB cells allow the monitoring of NF-κB activation by determining the activity of SEAP. The levels of NF-κB-induced SEAP in the cell culture supernatant are readily assessed with QUANTI-Blue™, a SEAP detection reagent.

THP1-Blue™ NF-κB cells are highly responsive to PRR agonists that trigger the NF-κB pathway (see figure 1). THP1-Blue™ NF-κB cells express all TLRs, as determined by RT-PCR, but respond only to ligands for TLR2, TLR1/2, TLR2/6, TLR4, TLR5 and TLR8.

THP1-Blue™ NF-κB cells are resistant to the selectable marker Blasticidin.

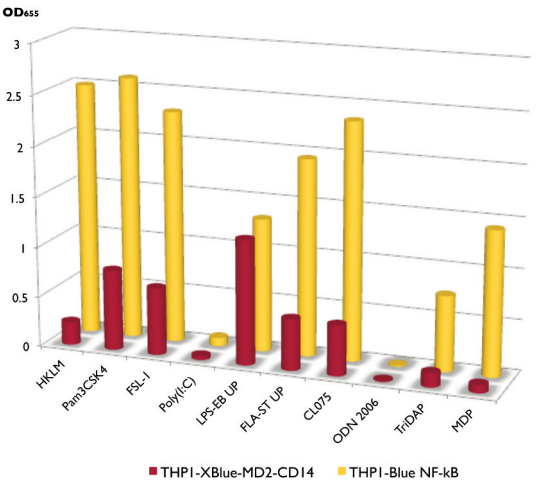

Figure 1: NF-κB response of THP1-Blue™ NF-κB cells. Cells were incubated with 10^7 cells/ml HKLM (TLR2), 10 ng/ml Pam3CSK4 (TLR1/2), 1 ng/ml FSL-1 (TLR2/6), 10 µg/ml poly(I:C) (TLR3), 100 ng/ml LPS-EB Ultrapure (TLR4), 100 ng/ml FLA-ST UP (TLR5), 3 µg/ml CL075 (TLR8), 10 µg/ml ODN2006 (TLR9), 10 µg/ml Tri-DAP (NOD1) or 10 µg/ml MDP (NOD2). After 24h incubation, the levels of NF-κB-induced SEAP were assessed from the cell culture supernatant using QUANTI-Blue™.

PRODUCT INFORMATION

Contents and Storage

- 1 vial of THP1-Blue™ NF-κB cells (5-7 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 Blasticidin (10 mg/ml). Store Blasticidin at 4°C for 6 months, or at -20°C for up to 12 months.
- 1 pouch of QUANTI-Blue™. Store QUANTI-Blue™ pouch at room temperature for 6 months. Reconstituted QUANTI-Blue™ medium is stable 2 weeks at 4°C. Keep reconstituted QUANTI-Blue™ 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.

Product Warranty

InvivoGen warrants that cells shall be viable upon shipment from InvivoGen for a period of thirty days, provided they have been properly stored and handled during this period.

Handling Cells Upon Arrival

We strongly recommend that you propagate the cells, using the provided procedure, as soon as possible. This will ensure the best cell viability and assay performance. 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-Blue™ 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 TLR8 responses are robust. Responses to TLR3, TLR7 and TLR9 are hardly detectable even when high concentrations of the cognate ligands are used.
THP1-Blue™ NF-κB cells respond to NOD1 and NOD2 agonists.

THP1-Blue™ 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.

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SAFETY CONSIDERATIONS

Biosafety Level 1

HANDLING PROCEDURES

Required Cell Culture Medium

• **Growth Medium:** RPMI 1640 (2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES and 1.0 mM sodium pyruvate) with 10% heat-inactivated fetal bovine serum (30 min at 56°C), 100 µg/ml Normocin™, Pen-Strep (50 U/ml-50 µg/ml)

Note:

- The amount of glucose in the growth medium can be reduced to 2 g/L which may result in slower cell growth.

- 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, or IMDM (Iscove's Modified Dulbecco's Media), 20% FBS, 5% DMSO

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 cm^2 tissue culture flask containing 5 ml of growth medium.

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 (Nalgene) 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 and are growing well (after at least one passage), maintain and subculture the cells in growth medium supplemented with 10 µg/ml of Blasticidin.

2- Pass the cells every 3 days by inoculating 7 x 10^5 cells/ml. Do not allow the cell concentration to exceed 2 x 10^6 cells/ml.

Note: To ensure the best results:

- Use THP1-Blue™ 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.

DETECTION OF TLR STIMULATION

Positive Control Preparation (HKLM)

1- Prepare a 10^9 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.

- Samples containing a phosphatase activity cannot be tested as they can result in false positive results (like serum not previously heat-inactivated).

TLR Assay

1 -Centrifuge cells at 1000-1500 RPM (RCF 200 - 300 g) for 5 minutes.

2- Remove supernatant and resuspend THP1-Blue™ NF-κB cells at 5 x 10^5 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 CO_2 incubator for 18-24 h.

6- Prepare QUANTI-Blue™ following the instructions on the pouch.

7- Add 180 µl of resuspended QUANTI-Blue™ per well of a flat-bottom 96-well plate.

8- Add 20 µl of THP1-Blue™ NF-κB cells supernatant.

9- Incubate the plate at 37°C incubator for 1-8 h.

10- Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Description	Catalog Code
Blasticidin	Selective antibiotic	ant-bl-1
CL075	TLR8 ligand	tlrl-c75
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-Blue™	SEAP detection medium	rep-qb1
TLR RT-Primer Set	RT-PCR primers	rts-htlrs
Tri-DAP	NOD1 ligand	tlrl-tdap

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