THP1-Blue™ KI-IP10 Cells

CXCL10 Knockin Lucia luciferase gene and NF-κB SEAP Reporter Monocytes

Catalog code: thpb-ip10kilc

https://www.invivogen.com/thp1-blue-ki-ip10

For research use only

Version 20J26-MM

PRODUCT INFORMATION

Contents and Storage

- **1 vial of THP1-Blue™ KI-IP10 cells** (3-7 x 10^6 cells)

IMPORTANT: Cells are shipped frozen. If cells are not frozen upon arrival, contact InvivoGen immediately.

- **1 ml of Blasticidin** (10 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 pouch of QUANTI-Luc™** (Lucia luciferase detection reagent). Store pouch at -20 °C. Reconstituted QUANTI-Luc™ is stable for 1 week at 4 °C and for 1 month at -20 °C. Protect from light.
- **1 ml of QB reagent** and **1 ml of QB buffer** (sufficient to prepare **100 ml of QUANTI-Blue™ Solution,** a SEAP detection reagent). Store QB reagent and QB buffer at -20 °C. QUANTI-Blue™ Solution is stable for 2 weeks at 4 °C and for 2 months at -20 °C.

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

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.

Cell Line Stability

Cells will undergo genotypic changes over time resulting in reduced responsiveness 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. THP1-Blue™ KI-IP10 cells should not be passaged more than 20 times to remain fully functional.

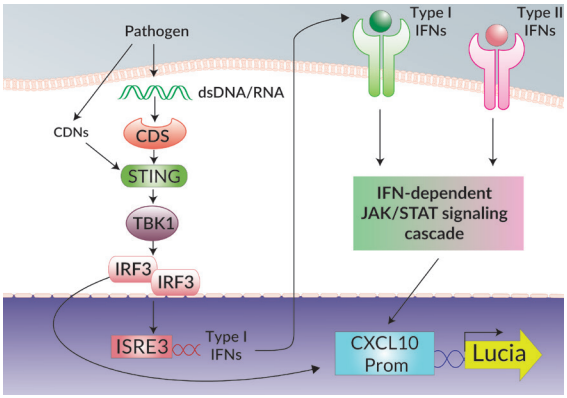
Quality Control

- Lucia luciferase gene knockin for IP10/CXCL10 was verified by PCR and DNA sequencing.
- THP1-Blue™ KI-IP10 cells have been stimulated with and respond to type I IFNs as well as various agonists of STING, Toll-like receptors (TLR) and RIG-I-like receptors (RLRs).
- The stability for 20 passages following thawing has been verified.
- THP1-Blue™ KI-IP10 cells are guaranteed mycoplasma-free.

BACKGROUND

Interferon-inducible protein-10 (IP10), more commonly known as CXCL10 (C-X-C motif ligand 10), belongs to a family of chemokines that includes CXCL9 and CXCL11. Canonically, CXCL10 is identified as an interferon stimulated gene (ISG) due to its induction by the JAK/STAT signaling cascades activated by type I and II IFNs[1]. Additionally, CXCL10 is strongly induced by the activation of the STING/TBK1/IRF3 signaling axis.

This cytosolic DNA-sensing pathway is activated by recognition of pathogen-derived nucleic acids and cyclic dinucleotides (CDNs) by cytosolic DNA sensors (CDS) and STING, respectively[2]. CXCL10 expression parallels the host's pro-inflammatory response to a number of pathogens including viruses such as respiratory syncytial virus, bacteria such as *Helicobacter pylori*, and parasites such as *Plasmodium falciparum*[3]. Therefore, CXCL10 expression has been suggested as a biomarker of disease severity. CXCL10 is a pleiotropic molecule that acts on both innate and adaptive immune cells. These diverse actions make it capable of exerting potent biological functions including apoptosis and the regulation of cell proliferation in infectious and inflammatory diseases. Interestingly, CXCL10 inhibits angiogenesis and therefore exerts additional important anti-tumor effects[3].



1. Muller, M. *et al.*, 2010. The chemokine receptor CXCR3 and its ligands CXCL9, CXCL10 and CXCL11 in neuroimmunity-a tale of conflict and conundrum. Neuropathol Appl Neurobiol. 36:368-387. 2. Motani, K. *et al.*, 2015. DNA-Mediated Cyclic GMP-AMP Synthase-Dependent and -Independent Regulation of Innate Immune Responses. J Immunol. 194:4914-4923. 3. Liu, M. *et al.*, 2011. CXCL10/IP-10 in infectious diseases pathogenesis and potential therapeutic implications. Cytokine Growth Factor Rev. 22:121-130.

CELL LINE DESCRIPTION

THP1-Blue™ KI-IP10 cells have been engineered from the human monocyte cell line THP1-Blue™ NF-κB to examine specifically the endogenous expression of CXCL10 in response to bacterial and viral infection, while preserving its genomic environment. In these cells, the CXCL10 open reading frame has been replaced with a Lucia luciferase reporter gene (from ATG to TGA), maintaining its expression under the control of the endogenous CXCL10 promoter. This allows the specific study of CXCL10 expression upon stimulation with agonists such as nucleic acids and cyclic dinucleotides (CDNs). The induction of endogenous CXCL10 expression can be easily quantified by assessing the expression of Lucia luciferase using the detection reagent QUANTI-Luc™. Furthermore, the activation of the NF-κB pathway, using an agonist such as tumor necrosis factor-α (TNF-α), is convenient for cross sample normalization by monitoring the activation of SEAP using the detection reagent QUANTI-Blue™.

THP1-Blue™ KI-IP10 cells are resistant to blasticidin.

TECHNICAL SUPPORT
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SAFETY CONSIDERATIONS

Biosafety Level 1

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** RPMI 1640, 10% (v/v) heat-inactivated fetal bovine serum (HI-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 differentiation of the monocytes and activation of the reporter gene.

- **Freezing Medium:** 90% FBS, 10% DMSO
- **Test Medium:** RPMI 1640, 10% heat-inactivated FBS, Pen-Strep (100 U/ml-100 µg/ml).

Required Selective Antibiotics

Blasticidin

Initial Culture Procedure

The first propagation of cells should be for generating stocks for future use. This ensures the ongoing stability and performance of the cells.

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 aseptic conditions.

3. Transfer the cells to a tube 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 cells at 150 x g (RCF) for 10 mins.
5. Remove the supernatant containing the cryoprotective agent and resuspend the cells with 1 ml of growth medium (with 20% heat-inactivated FBS).
6. Transfer the cells (1 ml) 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 and 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells to a density of 5-7 x 10^6 cells/ml in freshly prepared freezing medium.
2. Dispense 1 ml aliquots of the cell suspension into cryogenic vials.
3. Place the vials in a freezing container and store at -80°C overnight.
4. Transfer the 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 two passages), subculture the cells in growth medium (with 10% heat-inactivated FBS). To maintain selection pressure, add 10 µg/ml of Blasticidin to the growth medium every other passage.
2. Passage 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

- Use THP1-Blue™ KI-IP10 cells with less than 20 passages.

- Handling of cells should be as short as possible to prevent any damage resulting from a prolonged stay at room temperature without 5% CO_2

REPORTER ASSAYS

Cell Suspension Preparation

1. Centrifuge at 150 x g (RCF) for 10 mins or 300 x g (RCF) for 5 mins.
2. Remove supernatant and resuspend THP1-Blue™ KI-IP10 cells to 5 x 10^5 cells/ml in freshly prepared test medium.

Note: It is recommended to perform the assay with test medium, which does not contain Normocin™ or Blasticidin.

Detection of IP10 (CXCL10) induction

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

1. Add 20 µl of sample per well of a flat-bottom 96-well plate including 2'3' cGAMP as a positive control and endotoxin free water as a negative control (use new tips for each well to avoid cross-contamination).
2. Add 180 µl of the cell suspension (~100,000 cells) per well.
3. Incubate the plate for 18-24 h at 37°C and 5% CO_2.
4. Prepare QUANTI-Luc™ following the instructions on the enclosed data sheet.
5. Setup 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. Add 10 µl of the THP1-Blue™ KI-IP10 cells supernatant into a 96-well white (opaque) or black plate, or a luminometer tube.
7. Prime the injector with blank QUANTI-Luc™ assay solution and proceed with the measurement.

Detection of NF-κB stimulation

1. Add 20 µl of sample per well of a flat-bottom 96-well plate, including a positive control (such as TNF-α) and endotoxin free water as a negative control (use new tips for each well to avoid cross-contamination).
2. Add 180 µl of the cell suspension (~100,000 cells) per well.
3. Incubate the plate for 18-24 h at 37°C and 5% CO_2.
4. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed data sheet.
5. Add 180 µl of reconstituted QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
6. Add 20 µl of the THP1-Blue™ KI-IP10 cells supernatant.
7. Incubate the plate at 37°C for 1-6 h.
8. Determine SEAP levels using a spectrophotometer at 620-655 nm.

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.

RELATED PRODUCTS

Product	Description	Cat. Code
2'3'-cGAMP	STING ligand	tlrl-nacga23
2'3'-c-di-AM(PS)$_2$ (Rp,Rp)	STING ligand	tlrl-nacda2r
3p-hpRNA	RIG-I ligand	tlrl-hprna
Ruxolitinib	JAK1/2 inhibitor	tlrl-rux
Rec hTNF-α	Recombinant human cytokine	rcyc-htnfa
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs1
QUANTI-Luc™	Lucia detection reagent	rep-qlc1
Blasticidin	Selective antibiotic	ant-bl-1
Normocin™	Selective antibiotic	ant-nr-1
Lyovec™	Transfection reagent	lyec-12

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