HEK-Blue™ mTLR3 Cells

SEAP Reporter 293 cells expressing the mouse TLR3 gene

Catalog # hkb-mtlr3

For research use only

Version # 16I16-MM

PRODUCT INFORMATION

Contents and Storage

• 1 vial of HEK-Blue™ mTLR3 Cells (5-7 x 10^6 cells) in freezing medium
IMPORTANT: Cells are shipped frozen. If cells are not frozen upon arrival, contact InvivoGen immediately.
• 100 µl Blasticidin selective antibiotic (10 mg/ml). Store at 4 °C or at -20 °C.*
• 100 µl Zeocin™ selective antibiotic (100 mg/ml). Store at 4 °C or at -20 °C.*
• 1 ml Normocin™ (50 mg/ml). Normocin™ is 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 HEK-Blue™ Detection, a cell culture medium (50 ml) for real-time detection of SEAP. Store pouch at 4 °C for 6 months. Reconstituted HEK-Blue™ Detection is stable for 2 weeks at 4 °C. Protect from light.
Note: Data sheets for all components are available on our website.

Handling Cells Upon Receipt

Cells must be thawed **immediately** upon receipt and grown according to handling procedures (as described overleaf), to ensure cell viability and proper assay performance.
*Note: **Do not freeze the 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 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. HEK-Blue™ mTLR3 cells should not be passaged more than 20 times to remain fully efficient.
HEK-Blue™ mTLR3 cells should be maintained in growth medium in the presence of blasticidin and Zeocin™. Antibiotic pressure with blasticidin is required to maintain the plasmid coding for mTLR3, and Zeocin™ is required to maintain the plasmid coding for SEAP.

Quality Control

• HEK-Blue™ mTLR3 cells have been stimulated by various pathogen recognition receptor (PRR) agonists. As expected, TLR3 agonists induced the production of SEAP.
• Expression of the murine TLR3 gene has been confirmed by RT-PCR.
• These 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

TLR3 recognizes double-stranded RNA (dsRNA), a molecular pattern associated with viral infection. Stimulation with poly(I:C), a synthetic analog of dsRNA, was shown to induce hyporesponsiveness in TLR3-deficient mice and considerable responsiveness in HEK293 cells expressing TLR3[1], suggesting a specific recognition to poly(I:C) by TLR3. TLR3 signals mainly through a MyD88-independent pathway involving the TRIF/TICAM1 adapter protein that leads to the production of IFN-ß and causes dendritic cells to mature[2].

1. Alexopoulou L. *et al.*, 2001. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature, 413(6857):732-8. **2. Yamamoto M. *et al.* 2002.** Cutting edge: A novel Toll/Il-1 receptor domain-containing adapter that preferentially activates the IFN-ß promoter in the Toll-like receptor signaling. J Immunol, 169(12):6668-6672.

PRODUCT DESCRIPTION

HEK-Blue™ mTLR3 Cells are designed for studying the stimulation of mouse TLR3 (mTLR3) by monitoring the activation of NF-κB. HEK-Blue™ mTLR3 Cells were obtained by co-transfection of the mTLR3 gene and an optimized secreted embryonic alkaline phosphatase (SEAP) reporter gene placed under the control of an NF-κB and AP-1-inducible promoter into HEK293 cells. Stimulation with a TLR3 ligand activates NF-κB and AP-1 which induce the production of SEAP.
Levels of SEAP can be easily determined with HEK-Blue™ Detection, a cell culture medium that allows for real-time detection of SEAP. HEK-Blue™ Detection is a one-step procedure and extremely simple to use. It is applicable to high-throughput screening. HEK-Blue™ Detection contains all the nutrients necessary for cell growth and a specific SEAP color substrate. The hydrolysis of the substrate by SEAP produces a purple/blue color that can be easily detected with the naked eye or measured with a spectrophotometer.
SEAP activity can also be assessed using the alkaline phosphatase detection reagent, QUANTI-Blue™. With the QUANTI-Blue™ assay, cells are stimulated in a culture medium containing heat-inactivated fetal bovine serum. Following cell activation, QUANTI-Blue™ is used to detect SEAP in the cell supernatant. This colorimetric assay allows the same cell cultures to be repeatedly sampled for kinetic studies or further experimentation.
For more information, visit http://www.invivogen.com/quanti-blue

HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1.
Note: The parental cell line for HEK-Blue™ mTLR3 cells is HEK-Blue™ Null1-k cells (SEAP reporter cells; expression levels of TLR3 are 100-fold lower than in HEK-Blue™ mTLR3 Cells).

SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ mTLR3 cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) that require Biosafety Level 2 according to CDC guidelines. The biosafety level may vary depending on the country.

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

HANDLING PROCEDURES

Required Cell Culture Medium

• Growth Medium: DMEM, 4.5 g/l glucose, 10% (v/v) fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml Normocin™, 2 mM L-glutamine

• Freezing Medium: DMEM, 20% (v/v) fetal bovine serum, 10% (v/v) DMSO

Required Selective Antibiotic(s)

• Blasticidin and 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% (v/v) ethanol.
Note: All steps from this point should be carried out under strict aseptic conditions.
3- Transfer cells in a larger vial containing 15 ml of pre-warmed growth medium. **Do not add selective antibiotics until the cells have been passaged twice.**
4- Centrifuge vial at 1000-1200 RPM (RCF 200-300 g) for 5 minutes.
5- Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium without selective antibiotics.
6- Transfer the vial contents to a 25 cm^2 tissue culture flask containing 5 ml of growth medium without selective antibiotics.
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 freshly prepared with cold growth medium.
Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.
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- Maintain and subculture the cells in growth medium supplemented with 10 µg/ml of blasticidin and 100 µg/ml of Zeocin™.
2- Renew growth medium twice a week.
3- Cells should be passaged when a 70-80% confluency is reached, detach the cells in presence of PBS by tapping the flask or by using a cell scraper. Do not let the cells grow to 100% confluency.
Note: The response of HEK-Blue™ mTLR3 cells can be altered by the action of trypsin. Do not use trypsin to detach HEK-Blue™ mTLR3 cells.

TLR3 Stimulation determined using HEK-Blue™ Detection

HEK-Blue™ Detection is a cell culture medium that allows the detection of SEAP as the reporter protein is secreted by the cells. Prepare HEK−Blue™ Detection following the instructions on the enclosed data sheet.
Note: Before the test, the cells should be 50-80% confluent.

1- Add 20 µl of each sample per well of a flat-bottom 96-well plate.
2- Add 20 µl of a positive control (such as poly(I:C) HMW, 1 µg/ml) in one well.
3- Add 20 µl of a negative control (such as sterile, endotoxin-free water) in one well.
4- Remove HEK-Blue™ mTLR3 cells from the incubator and discard growth medium.
5- Gently rinse cells with pre-warmed 5-10 ml PBS (for a T-75 flask).
6- Add 2-5 ml pre-warmed PBS (for a T-75 flask) and place the cells at 37 °C for 1- 2 min, detach the cells by tapping the flask or by using a cell scraper. Dissociate cell clumps by gently pipetting up and down.
Note: Do not use trypsin to detach HEK-Blue™ mTLR3 cells.
7- Count cells which have been resuspended in pre-warmed PBS.
Note: Avoid centrifugation of HEK-Blue™ mTLR3 cells.
8- Prepare a cell suspension ~280,000 cells per ml in HEK-Blue™ Detection medium and immediately add 180 µl of the cell suspension (~50,000 cells) per well.
Note: Avoid prolonged incubation of cells at room temperature in HEK-Blue™ Detection medium as it may lead to high background or false positive readings.
9- Incubate the plate at 37 °C in 5% CO_2 for 6-16 h. SEAP can be observed with naked eye and determined using a spectrophotometer at 620-655 nm.

Specificity of HEK-Blue™ mTLR3 Cells

As HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1, HEK-Blue™ mTLR3 Cells will respond to their cognate ligands, such as poly(I:C), flagellin and C12-iE-DAP, respectively. In order to identify TLR3-specific responses, we recommend to use HEK-Blue™ Null1-k cells as a control cell line. In non-saturating conditions, the response to TLR3 ligands in HEK-Blue™ mTLR3 cells is normally between 10 and 100-fold higher than in HEK-Blue™ Null1-k cells.
Note: HEK-Blue™ mTLR3 cells may be stimulated in a TLR3-independent manner as NF-κB/AP-1 can be activated by a wide variety of stimuli (e.g. TNF-α and PMA).

RELATED PRODUCTS

Product	Catalog Code
Blasticidin	ant-bl-1
HEK-Blue™ Detection	hb-det2
HEK-Blue™ Null1-k Cells	hkb-null1k
Normocin™	ant-nr-1
Poly(I:C) HMW (TLR3 ligand)	tlrl-pic
Poly(I:C) LMW (TLR3 ligand)	tlrl-picw
Poly(A:U) (TLR3 ligand)	tlrl-pau
QUANTI-Blue™	rep-qb1
Zeocin™	ant-zn-1

HEK-Blue™ Detection

Cell culture medium for the real-time detection of secreted alkaline phosphatase

Catalog # hb-det2, hb-det3

For research use only

Version # 16C07-MM

PRODUCT INFORMATION

Contents:

HEK-Blue™ Detection is provided in sealed pouches and is available in two quantities:

- hb-det2: 5 pouches
- hb-det3: 10 pouches

Each pouch contains everything needed to prepare 50 ml of medium for the colorimetric detection of secreted embryonic alkaline phosphatase (SEAP).

Storage and stability:

- Store sealed pouches at 2-8 °C. Product is stable for 6 months at 2-8 °C in unopened pouches.

Important: *The correct storage temperature for this product is 2-8 °C (some pouches may be mislabeled).*

- Reconstituted HEK-Blue™ Detection is stable for 2 weeks at 2-8 °C and for 2 months at -20 °C. Protect from light.

DESCRIPTION

HEK-Blue™ Detection is a cell culture medium developed to provide a fast and convenient method to monitor SEAP expression. Detection of SEAP occurs as the reporter protein is secreted by the cells grown in HEK-Blue™ Detection. HEK-Blue™ Detection changes to a purple/blue color in the presence of alkaline phosphatase activity.

SEAP is a widely used reporter gene. It is a truncated form of placental alkaline phosphatase, a GPI-anchored protein. SEAP is secreted into cell culture supernatant and therefore offers many advantages over intracellular reporters. It allows to determine reporter activity without disturbing the cells, does not require the preparation of cell lysates and can be used for kinetic studies. Using HEK-Blue™ Detection, SEAP expression can be observed visually, and unlike flourescent or luminescent reporters can be easily quantified using a microplate reader or spectrophotometer.

HEK-Blue™ Detection is applicable to high-throughput screening.

METHODS

Preparation of HEK-Blue Detection:

1. Pour the contents of one pouch of HEK-Blue™ Detection in a sterile vial/bottle.
2. Solubilize the powder with 50 ml of endotoxin-free water.
3. Homogenize by vortexing or swirling the solution.
4. Warm reconstituted HEK-Blue™ Detection to 37 °C for 20 min to 1 hour.
5. Filter the medium on a 0.2 µm membrane into a sterile vial/bottle.
6. Keep the HEK-Blue™ Detection medium at 37 °C before use or store at 2-8 °C for up to 2 weeks.

Detection of SEAP activity

The following protocol refers to the use of 96-well plates. Vary your procedure accordingly depending on volumes of reagents needed based on the size of your wells.

1. Prepare cell suspension:
   - detach cells and resuspend in a small volume of PBS
   - count cells
   - add appropriate amount of PBS-resuspended cells in HEK-Blue™ Detection to obtain a cell suspension at the expected concentration.
2. Add 20 µl of SEAP-inducer compound or negative control (such as PBS) per well.
3. Add 180 µl of cell suspension per well.

Note: To obtain more consistent results, we recommend to mix SEAP-inducer and cell suspension by pipetting up and down.

4. Incubate overnight at 37 °C, in 5% CO2.
5. Assess SEAP activity with the naked eye or by reading the optical density (OD) at 620-655 nm with a microplate reader.

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ LPS Detection Kit	rep-lps
HEK-Blue™ TLR cells	hkb-tlr
PlasmoTest™	rep-pt2
QUANTI-Blue™	rep-qb

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