HEK-Blue™ hTLR3 Cells

SEAP reporter 293 cells expressing the human TLR3 gene

Catalog code: hkb-htlr3

https://www.invivogen.com/hek-blue-htlr3

For research use only

Version 23E15-MM

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10^6 of HEK-Blue™ hTLR3 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 Blasticidin** (10 mg/ml), store at 4 °C or at -20 °C.*
- **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 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 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'.

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™ hTLR3 should not be passaged more than 20 times to remain fully efficient.

Quality Control

- HEK-Blue™ hTLR3 cells have been stimulated by various pathogen recognition receptor (PRR) agonists. As expected, TLR3 agonists induced the production of SEAP.
- The expression of human TLR3 (hTLR3) in this cell line has been confirmed by RT-PCR.
- Cell line stability for 20 passages following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

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-β and causes dendritic cells to mature[2].

1. Alexopoulou L. *et al.*, 2001. Recognition of double-stranded RNA and activation of NFkappaB 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 IFNß promoter in the Toll-like receptor signaling. J Immunol, 169(12):6668-6672.

PRODUCT DESCRIPTION

HEK-Blue™ hTLR3 cells are designed for studying the stimulation of human TLR3 (hTLR3) by monitoring the activation of NF-κB and AP-1. These cells are derived from the human embryonic kidney HEK293 cell line. HEK-Blue™ hTLR3 cells were obtained by co-transfection of the hTLR3 and SEAP (secreted embryonic alkaline phosphatase) reporter genes. The SEAP reporter gene is placed under the control of the IFN-β minimal promoter fused to five NF-κB and AP-1-binding sites.

Stimulation with a TLR3 ligand activates NF-κB and AP-1 which induce the production of SEAP. The levels of SEAP can be easily determined with HEK-Blue™ Detection, a cell culture medium that allows real-time detection of SEAP. HEK-Blue™ Detection is a one-step procedure and is applicable to high-throughput screening. SEAP activity can also be assessed using the alkaline phosphatase detection reagent, QUANTI-Blue™. This assay allows the same cell cultures to be repeatedly sampled for kinetic studies or further experimentation. For more information, visit https://www.invivogen.com/quanti-blue.

HEK-Blue™ hTLR3 cells are resistant to blasticidin and Zeocin®. Of note, HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1.

Note: The parental cell line for HEK-Blue™ hTLR3 cells is HEK-Blue™ Null1 cells (SEAP reporter cells; expression levels of hTLR3 are 100-fold lower than in HEK-Blue™ hTLR3 Cells).

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SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ hTLR3 cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) that require Biosafety Level 2 according to the American Center for Disease Control and Prevention (CDC) guidelines. The biosafety level may vary depending on the country. For example, in Germany HEK293 cell lines are designated Biosafety Level 1 according to the Central Committee of Biological Safety, Zentrale Kommission für die Biologische Sicherheit (ZKBS). Please check with your country's regulatory authority regarding the use of these cells.

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% (v/v) fetal bovine serum (FBS), Pen-Strep (100 U/ml-100 µg/ml), 100 µg/ml Normocin™
- **Freezing Medium:** DMEM with 20% FBS and 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 (approximately 2 minutes).
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 of the steps from this point should be carried out under strict aseptic conditions.

3. Transfer cells to a tube containing 15 ml of pre-warmed growth medium. **Do not add selection antibiotics until the cells have been passaged twice.**
4. Centrifuge tube at 300 x g (RCF) 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 contents to a T-25 tissue culture flask containing 5 ml of growth medium without selective antibiotics.
7. Place the culture at 37°C in 5% CO_2.

Note: Following thawing, adherence to the plastic surface and proliferation may be slow. In the initial culture procedure, these cells usually take 3-4 days before reaching confluency in a T-25 tissue culture flask.

Frozen Stock Preparation

1. Resuspend cells at 3-5 x 10^6 cells/ml in freshly prepared freezing medium.

Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.

2. Dispense 1 ml of the cell suspension 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. HEK-Blue™ hTLR3 cells grow as adherent cells. Detach the cells using trypsin for 2-3 mins at room temperature (RT).

Note: Prolonged action of trypsin or incubation at 37°C may alter the cell surface expression of receptors.

2. Maintain and subculture the cells in growth medium supplemented with 30 µg/ml of blasticidin and 100 µg/ml of Zeocin®.
3. Renew the growth medium twice a week.
4. Passage cells when 70-80% confluency is reached. Do not let the cell grow to 100% confluency.

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 test 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 (final concentration) in one well.
3. Add 20 µl of a negative control such as sterile, endotoxin-free water or growth medium in one well.
4. Remove 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 pre-warmed PBS (2-5 ml for a T-75 flask) and place the cells at 37°C for 1-2 min. Detach the cells by tapping the flask. Dissociate cell clumps by gently pipetting up and down.

Note: We recommend avoiding the use of trypsin to detach cells for the functional assays (see FAQs online).

7. Count cells which have been resuspended in pre-warmed PBS.

Note: For the reporter assay, avoid centrifugation of HEK-Blue™ hTLR3 cells.

8. Prepare a ~280,000 cells/ml suspension in HEK-Blue™ Detection medium and immediately dispense 180 µl of the cell suspension (~50,000 cells) per well.

Note: At this point in the protocol, care should be taken to avoid prolonged incubation of cells at room temperature in HEK-Blue™ Detection medium as this can 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 determined by reading the optical density at 620-655 nm using a spectrophotometer.

Specificity of HEK-Blue™ hTLR3 cells

As HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1, HEK-Blue™ hTLR3 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 cells as a control cell line. In non-saturating conditions, the response to TLR3 ligands in HEK-Blue™ hTLR3 cells is normally between 10 and 100-fold higher than in HEK-Blue™ Null1 cells.

Note: HEK-Blue™ hTLR3 cells may be stimulated in a TLR3-independent manner as NF-kB/AP-1 can be activated by a wide variety of stimuli (e.g. TNF-a and PMA).

RELATED PRODUCTS

Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
Poly(I:C) HMW	TLR3 agonist	tlrl-pic
HEK-Blue™ Detection	SEAP Detection medium	hb-det2
HEK-Blue™ Null1 Cells	Control cell line	hkb-null1
Normocin™	Antimicrobial reagent	ant-nr-1
Zeocin®	Selection antibiotic	ant-zn-1

HEK-Blue™ Detection

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

Catalog code: hb-det2, hb-det3

https://www.invivogen.com/hek-blue-detection

For research use only

Version 23L22-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. Unopened pouches are stable for at least 6 months when stored properly.
Important: For the exact expiry date please see the corresponding CoA.
- 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, which will change 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 the determination of 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 for high-throughput screening.

METHODS

Preparation of HEK-Blue™ Detection

1. Pour the contents of one pouch of HEK-Blue™ Detection into a sterile vial/bottle.
2. Solubilize the powder with 50 ml of cell culture grade water.
3. Vortex vigorously until powder is completely dissolved.
4. Warm reconstituted HEK-Blue™ Detection to 37 °C for at least 3 hours.
5. Filter the medium through a 0.2 µm bottle-top vacuum filter into a sterile vial/bottle.
Note: We recommend using filter units providing a large filter area to facilitate filtration.
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 is for the use of HEK-Blue™ Detection in 96-well plates. This will vary slightly depending on the volume of reagents needed, based on different plate sizes.

1. Prepare the cell suspension by detaching the cells and resuspending in a small volume of PBS.
2. Count the cells.
3. Add an appropriate amount of PBS-resuspended cells in HEK-Blue™ Detection to obtain a cell suspension at the expected concentration.
4. Add 20 µl of SEAP-inducer compound or negative control (such as PBS) per well.
5. Add 180 µl of cell suspension per well.
Note: To obtain more consistent results, we recommend to mix the SEAP-inducer and cell suspension by pipetting up and down.
6. Incubate overnight at 37 °C, in 5% CO_2.
7. Determine SEAP activity with the naked eye or by reading the optical density (OD) at 620-655 nm.

RELATED PRODUCTS

Product	Description	Cat. Code
pSELECT-zeo-SEAP	SEAP reporter gene	psetz-seap
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
Recombinant SEAP Protein	Control for SEAP assays	rec-hseap

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