

HEK-Blue™ hTLR4 Cells

SEAP Reporter 293 cells expressing the human TLR4 gene

Catalog code: hkb-htrl4

<https://www.invivogen.com/hek-blue-htrl4>

For research use only

Version 22B21-MM

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10⁶ HEK-Blue™ hTLR4 cells in a cryovial or shipping flask.

IMPORTANT: If cells provided in a cryovial are not frozen upon arrival, contact InvivoGen immediately.

- 2 x 1 ml HEK-Blue™ Selection (250X concentrate). A solution containing several selection antibiotics. 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 sealed pouches at 2-8 °C. Unopened pouches are stable for at least 6 months when stored properly. Reconstituted HEK-Blue™ Detection is stable for 2 weeks at 2-8 °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™ hTLR4 cells should not be passaged more than 20 times to remain fully efficient.

Quality Control

- HEK-Blue™ hTLR4 cells have been stimulated by various pathogen recognition receptor (PRR) agonists. As expected, TLR4 agonists induced the production of SEAP.
- The expression of human TLR4 and MD-2/CD14 genes has been confirmed by RT-PCR.
- The cell surface expression of human TLR4 in this cell line has been validated using fluorescence-activated cell sorting (FACS).
- The stability for 20 passages following thawing has been verified.
- 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 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.

BACKGROUND

Toll-like receptor 4 (TLR4) was the first TLR identified and is an important pattern recognition receptor (PRR) in innate immunity and inflammation. TLR4 is found both on the cell surface and in endosomes of innate immune cells including monocytes and macrophages, as well as on intestinal epithelium and endothelial cells¹. TLR4 primarily recognizes and is activated by lipopolysaccharide (LPS) and its toxic moiety Lipid A, a core component of Gram-negative bacteria². TLR4 does not directly interact with LPS and requires essential co-receptors, namely lipid-binding protein (LBP), MD-2, and CD14, to extract and deliver monomeric LPS to TLR4³. There are two distinct signaling cascades triggered by the dimerization of TLR4; the MyD88-dependent (at the cell surface) and TRIF-dependent (in endosomes) pathways. At the cell surface, activation of TLR4 initiates the MyD88-dependent pathway, ultimately leading to the 'early' activation of NF-κB and the production of a pro-inflammatory response³. Subsequently, the TLR4 complex can be endocytosed into endosomes and result in the 'late' activation of NF-κB⁴. TLR4 signaling is crucial in both acute and chronic inflammatory disorders and is thus an attractive target for novel treatments for conditions such as sepsis and cancer¹.

1. Ou T. *et al.*, 2018. The Pathologic Role of Toll-Like Receptor 4 in Prostate Cancer. *Front Immunol* 9:1188. 2. Cochet F. *et al.*, 2017. The Role of Carbohydrates in the Lipopolysaccharide (LPS)/Toll-Like Receptor 4 (TLR4) Signalling. *Int J Mol Sci.* 18. 3. Kuzmich N.N. *et al.*, 2017. TLR4 Signaling Pathway Modulators as Potential Therapeutics in Inflammation and Sepsis. *Vaccines (Basel)* 5. 4. Marongiu L. *et al.*, 2019. Below the surface: The inner lives of TLR4 and TLR9. *J Leukoc Biol* 106:147-60.

PRODUCT DESCRIPTION

HEK-Blue™ hTLR4 cells are designed for studying the stimulation of human TLR4 (hTLR4) by monitoring the activation of NF-κB. HEK-Blue™ hTLR4 cells were obtained by co-transfection of the hTLR4 gene, the MD-2/CD14 co-receptor genes, and a secreted embryonic alkaline phosphatase (SEAP) reporter gene into HEK293 cells. The SEAP reporter gene is placed under the control of the IL-12 p40 minimal promoter fused to five NF-κB and AP-1 binding sites. Stimulation with a TLR4 ligand activates NF-κB and AP-1 which induces 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.

HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1. Note: The parental cell line for HEK-Blue™ hTLR4 cells is HEK-Blue™ Null2 cells (SEAP reporter cells which do not express hTLR4).

HEK-Blue™ hTLR4 cells are resistant to Blasticidin, Hygromycin B and Zeocin®.

TECHNICAL SUPPORT

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Any questions about our cell lines?

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SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ hTLR4 cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) and thus may require Biosafety Level 2. The biosafety level varies by country. In the United States, HEK293 cell lines are designated Biosafety Level 2 according to the Center for Disease Control and Prevention (CDC). 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) heat-inactivated fetal bovine serum (FBS; 30 min at 56 °C), Pen-Strep (100 U/ml-100 µg/ml), 100 µg/ml Normocin™
- **Freezing Medium:** DMEM, 20% (v/v) FBS, 10% (v/v) DMSO

Required Selective Antibiotic(s)

- HEK-Blue™ Selection

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 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 vial contents to a 25 cm² tissue culture flask containing 5 ml of growth medium without selective antibiotics.
7. Place the culture at 37 °C in 5% CO₂.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10⁶ cells/ml in freezing medium freshly prepared with cold growth medium.
- Note: A T-75 culture flask typically yields enough cells for 3-4 frozen vials.*
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. HEK-Blue™ hTLR4 cells grow as adherent cells. Detach the cells in the presence of pre-warmed phosphate buffered saline (PBS) by tapping the flask or using trypsin for 2-3 min at room temperature (RT). *Note: The response of HEK-Blue™ hTLR4 cells can be altered by the prolonged action of trypsin. Do not incubate with trypsin at 37 °C and for no longer than 2-3 mins.*
2. Maintain and subculture the cells in growth medium supplemented with 1X HEK-Blue™ Selection.
3. Renew growth medium twice a week.
4. Cells should be passaged when a 70-80% confluency is reached. Do not let the grow to 100% confluency. *Note: The doubling time for the HEK-Blue hTLR4 cells is ~24 hours using the conditions described above.*

Cell Handling Recommendations

To ensure the best results:

- Use HEK-Blue™ hTLR4 cells with less than 20 passages.

REPORTER ASSAY

We recommend to use HEK-Blue™ hTLR4 cells with their corresponding parental cell line HEK-Blue™ Null2.

Note: For more information regarding the parental cell line please visit <https://www.invivogen.com/hek-blue-null2>.

1. Add 20 µl of each test sample per well of a 96-well flat-bottom plate. Include a positive control for both the parental and HEK-Blue™ hTLR4 cells (i.e. recombinant human TNF-α), as well as a negative control (i.e. culture medium only).
2. Add 20 µl of a TLR4 ligand such as LPS-EK Ultrapure, 100 ng/ml (final concentration) in a separate well.
3. Prepare a suspension of HEK-Blue™ hTLR4 and their parental HEK-Blue™ Null2 cells by gently rinsing the cells twice with pre-warmed phosphate buffered saline (PBS).
4. Detach the cells using by tapping the flask.

Notes for the reporter assay:

- Do not use trypsin to detach HEK-Blue™ hTLR4 cells.
 - Do not centrifuge HEK-Blue™ hTLR4 cells.
5. Count cells which have been resuspended in PBS.
 6. Prepare a cell suspension ~140,000 cells per ml in HEK-Blue™ Detection medium and immediately add 180 µl of the cell suspension (~25,000 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.*
 7. Incubate the plate at 37 °C in 5% CO₂ for 16-24 h. SEAP can be observed with naked eye and determined using a spectrophotometer at 620-655 nm.

Specificity of HEK-Blue™ hTLR4 Cells

As HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1, HEK-Blue™ hTLR4 cells will respond to their cognate ligands, such as poly(I:C), flagellin and C12-iE-DAP, respectively. In order to identify TLR4-specific responses, we recommend to use HEK-Blue™ Null2 cells as a control cell line.

Note: HEK-Blue™ hTLR4 cells may be stimulated in a TLR4-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	Description	Cat. Code
Anti-hTLR4-IgG	Neutralizing antibody	mabg-htlr4
HEK-Blue™ Detection	SEAP detection reagent	hb-det2
HEK-Blue™ Null2 Cells	Parental cells	hkb-null2
HEK-Blue™ Selection	Selection antibiotic mix	hb-sel
LPS-EB Ultrapure	LPS from <i>E. coli</i> O111:B4	tlr1-3pelps
LPS-EK Ultrapure	LPS from <i>E. coli</i> K12	tlr1-pek1ps
Normocin™	Antimicrobial reagent	ant-nr-1
Recombinant human TNF-α	Recombinant cytokine	rcyc-htnfa

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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 22C25-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 fluorescent 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 endotoxin-free water.
3. Swirl gently until powder is completely dissolved.
4. Warm reconstituted HEK-Blue™ Detection to 37°C for 30 minutes to 1 hour.
5. Filter the medium through a 0.2 µm membrane 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% CO₂.
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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