HEK-Blue™ hTLR2 Cells

SEAP Reporter 293 cells expressing the human TLR2 gene

Catalog # hkb-htlr2

For research use only

Version # 16I16-MM

PRODUCT INFORMATION

Contents and Storage

• 1 vial of HEK-Blue™ hTLR2 cells (3-7 x 10^6 cells) in freezing medium
IMPORTANT: Cells are shipped frozen. If cells are not frozen upon arrival, contact InvivoGen immediately.
• 2 x 1 ml HEK-Blue™ Selection (250X concentrate). A solution containing several selection antibiotics. Store HEK-Blue™ Selection 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™ hTLR2 cells should not be passaged more than 20 times to remain fully efficient.
HEK-Blue™ hTLR2 cells should be maintained in growth medium in the presence of Normocin™ (100 µg/ml) and 1X HEK-Blue™ Selection. Antibiotic pressure with HEK-Blue™ Selection is required to maintain the plasmid coding for hTLR2, CD14 and the plasmid coding for SEAP.

Quality Control

HEK-Blue™ hTLR2 cells have been stimulated by various pathogen recognition receptor (PRR) agonists. As expected, TLR2 agonists induced the production of SEAP. The expression of human TLR2 in this cell line has been validated using fluorescence-activated cell sorting (FACS). The expression of the human TLR2 and CD14 genes has been confirmed by RT-PCR. The stability of this cell line 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

TLR2 is involved in the recognition of a wide array of microbial molecules. TLR2 recognizes peptidoglycan, lipoteichoic acid and lipoprotein from gram-positive bacteria, lipoarabinomannan from mycobacteria, and zymosan from yeast cell wall. TLR2 cooperates with TLR6 in response to diacylated mycoplasmal lipopeptide[1], and associates with TLR1 to recognize triacylated lipopetides[2,3]. Simultaneous expression of the extracellular and intracellular domains of both TLR1 and TLR2 is essential for ligand recognition and subsequent ligand-induced signal activation[4]. Moreover, pathogen recognition by TLR2 is strongly enhanced by CD14[5].

1. Girard R *et al.*, 2003. Lipopolysaccharides from Legionella and Rhizobium stimulate mouse bone marrow granulocytes via Toll-like receptor 2. J Cell Sci. 116:293-302. **2. Ozinsky A. *et al.*, 2000.** The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. PNAS. 97:13766-71. **3. Thakran S. *et al.*, 2008.** Identification of Francisella tularensis lipoproteins that stimulate the Toll-like receptor (TLR) 2/TLR1 heterodimer. J Biol Chem 283: 3751-9. **4. Sandor F. *et al.*, 2003.** Importance of extra- and intracellular domains of TLR1 and TLR2 in NFkB signaling. J Cell Biol. 162: 1099-10. **5. Lotz S. *et al.*, 2004.** Highly purified lipoteichoic acid activates neutrophil granulocytes and delays their spontaneous apoptosis via CD14 and TLR2. J Leukoc Biol. 75(3):467-77.

PRODUCT DESCRIPTION

HEK-Blue™ hTLR2 Cells are designed for studying the stimulation of human TLR2 (hTLR2) by monitoring the activation of NF-κB. HEK-Blue™-hTLR2 Cells were obtained by co-transfection of the hTLR2 and SEAP (secreted embryonic alkaline phosphatase) reporter genes into HEK293 cells. 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. Additionally, the CD14 co-receptor gene was transfected into these cells to enhance the TLR2 response. Stimulation with a TLR2 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 TLR1, TLR3, TLR5, TLR6 and NOD1.
Note: The parental cell line for HEK-Blue™ hTLR2 Cells is HEK-Blue™ Null1 (SEAP reporter cells which do not express hTLR2).

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FAQ Any questions about our cell lines?
Visit our FAQ page.

InvivoGen
www.invivogen.com

SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ hTLR2 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.

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)

- 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 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- Prepare 1 ml aliquots of cells in 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 1X HEK-Blue™ Selection.
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™ hTLR2 cells can be altered by the action of trypsin. Do not use trypsin to detach HEK-Blue™ hTLR2 cells.

TLR2 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 FSL-1, 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™ hTLR2 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™ hTLR2 cells.
7- Count cells which have been resuspended in pre-warmed PBS. *Note: Do not centrifuge HEK-Blue™ hTLR2 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: 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 it can lead to high background or false positive readings.
9- Incubate the plate at 37°C in 5% CO_2 for 16-24 h. SEAP can be observed with naked eye and determined using a spectrophotometer at 620-655 nm.

Specificity of HEK-Blue™ hTLR2 Cells

As HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1, HEK-Blue™ hTLR2 cells will respond to their cognate ligands, such as poly(I:C), flagellin and C12-iE-DAP, respectively. In order to identify TLR2-specific responses, we recommend to use HEK-Blue™ Null1 cells as a control cell line. Furthermore, an anti-hTLR2 neutralizing antibody can be used to ensure the specificity of the TLR2 response.
Note: HEK-Blue™ hTLR2 cells may be stimulated in a TLR2-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
Anti-hTLR2-IgA	maba2-htlr2
FSL-1 (TLR2/6 ligand)	tlrl-fsl
HEK-Blue™ Detection	hb-det2
HEK-Blue™ Null1 Cells	hkb-null1
HEK-Blue™ Selection	hb-sel
HKLM (TLR2 ligand)	tlrl-hklm
MAb-hTLR2	mab-htlr2
Normocin™	ant-nr-1
PAb-hTLR2	pab-hstlr2
Pam3CSK4 (TLR1/2 ligand)	tlrl-pms
QUANTI-Blue™	rep-qb1

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 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 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% 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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