HEK-Blue™ hTLR2-TLR1 Cells

SEAP reporter 293 cells expressing the human TLR2 and TLR1 genes

Catalog code: hkb-htlr21

https://www.invivogen.com/hek-blue-htlr2tlr1

For research use only

Version 23K20-MM

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10^6 of HEK-Blue™ hTLR2-TLR1 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 the required 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 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™ hTLR2-TLR1 should not be passaged more than 20 times to remain fully efficient.

Quality Control

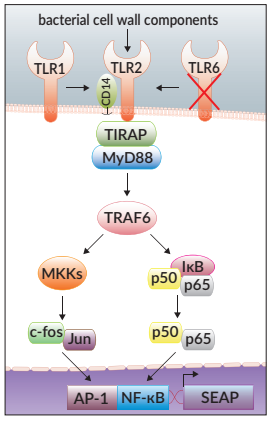
- HEK-Blue™ hTLR2-TLR1 cells have been stimulated by various pathogen recognition receptor (PRR) agonists. As expected, TLR2-TLR1 agonists induced the production of SEAP.
- The expression of human TLR2 (hTLR2) and CD14 in this cell line has been validated using fluorescence-activated cell sorting (FACS).
- The expression of hTLR1 has been confirmed by qRT-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



TLR2 plays a pivotal role in detecting a diverse range of pathogen-associated molecular patterns (PAMPs) from bacteria, viruses, fungi, and parasites[1]. Specifically, it recognizes cell-wall components including lipoproteins and peptidoglycan from bacteria, lipoteichoic acid from Gram$^+$ bacteria, lipoarabinomannan from mycobacteria, and zymosan from fungi. Notably, at the cell surface TLR2 forms a heterodimer with co-receptors TLR1 or TLR6, depending upon either tri- or diacylation of the ligand, respectively. Downstream signaling of both TLR2 heterodimers has been shown to be enhanced when in association with CD14[2]. Once a ligand binds to either TLR2-TLR1 or TLR2-TLR6, a MyD88-dependent activation of NF-κB and AP-1 occurs, ultimately leading to an innate immune response. Importantly, the ability for TLR2 to form heterodimers not only expands the range of PAMPs that it recognizes, but can also lead to divergent responses depending on the heterodimer involved[3]. To date, TLR2 alone as a functional homodimer has only been proposed, with no evidence to prove that it triggers a signaling cascade[1].

1. Oliveira-Nascimento L. *et al.*, 2012. The Role of TLR2 in Infection and Immunity. Front Immunol. 3:79. 2. 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. 3. Nguyen MT. *et al.*, 2017. Lipid moieties on lipoproteins of commensal and non-commensal staphylococci induce differential immune responses. Nat Commun. 8(1):2246.

HEK-BLUE™ TLR2 CELL COLLECTION

InvivoGen provides a collection of engineered HEK293-derived cell lines designed for investigating the TLR1-TLR2 and/or TLR2-TLR6 signaling pathways, by monitoring the activation of NF-κB. This collection was generated using HEK-Blue™ hTLR2 cells, which endogenously express TLR1 and TLR6 and have been stably transfected with human TLR2 and CD14 genes, together with an NF-κB/AP-1-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. Cells in the collection include HEK-Blue™ hTLR2 KO-TLR1/6 which harbors a double knockout of TLR1 and TLR6, as well as HEK-Blue™ hTLR2-TLR1 and HEK-Blue™ hTLR2-TLR6 derived from the double knockout which stably express exogenous human TLR1 and TLR6, respectively. The cell lines in this collection can be used independently or in combination to screen and validate ligands of both TLR2 heterodimers.

CELL LINE NAME	TLR1	TLR2	TLR6
HEK-Blue™ hTLR2	+	+	+
HEK-Blue™ hTLR2 KO-TLR1/6	-	+	-
HEK-Blue™ hTLR2-TLR1	+	+	-
HEK-Blue™ hTLR2-TLR6	-	+	+

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

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PRODUCT DESCRIPTION

HEK-Blue™ hTLR2-TLR1 cells are designed for studying the human TLR2-TLR1 heterodimer by monitoring the activation of NF-κB and AP-1. This cell line was generated by stable transfection of the hTLR1 gene into the HEK-Blue™ hTLR2 KO-1/6 cell line, an HEK293-derived cell line that overexpresses hTLR2 and CD14 with a double knockout of TLR1 and TLR6. Furthermore, HEK-Blue™ hTLR2-TLR1 cells stably express the SEAP reporter gene, under the control of the IFN-β minimal promoter fused to five NF-κB and AP-1 binding sites. Binding of a triacylated lipoprotein, such as Pam_3CSK_4, to the TLR2-TLR1 heterodimer activates NF-κB and AP-1 triggering 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™ Solution. 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™ hTLR2-TLR1 cells are resistant to blasticidin, hygromycin and Zeocin®. Of note, HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1.

SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ hTLR2-TLR1 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, 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)

- 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 (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 HEK-Blue™ Selection 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. Maintain and subculture the cells in growth medium supplemented with 1x HEK-Blue™ Selection.
2. Renew the growth medium twice a week.
3. Passage cells when 70-80% confluency is reached. Do not let the cell grow to 100% confluency.

TLR2-TLR1 Stimulation determined using HEK-Blue™ Detection

Prepare HEK-Blue™ Detection following the instructions on the enclosed data sheet. Use HEK-Blue™ hTLR2-TLR1 with their corresponding parental cell line, HEK-Blue™ hTLR2 KO-TLR1/6 cells as the control.

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 recombinant hIL-1β at 1 ng/ml (final concentration) in one well.
3. Add 20 µl of a TLR2-TLR1 ligand such as Pam_3CSK_4 at 10 ng/ml (final concentration) in one well.
4. Add 20 µl of a negative control such as ODN 2006 (TLR9 ligand) at 10 µg/ml (final concentration) or growth medium in one well.
5. Remove cells from the incubator and discard growth medium.
6. Gently rinse cells with pre-warmed 5-10 ml PBS (for a T-75 flask).
7. Detach cells with 0.08% trypsin-EDTA (diluted with PBS) for 2-5 minutes. Once cells appear detached add 2 volumes (approx. 10 ml for a T-75 flask) of pre-warmed growth media (containing 10 % FBS) to inactivate trypsin. Dissociate cell clumps by gently pipetting up and down.
8. Transfer the cell suspension to a tube and centrifuge at 300 x g (RCF) for 5 minutes.
9. Discard the supernatant, gently resuspend the cell pellet in pre-warmed PBS. Count cells which have been resuspended in pre-warmed PBS.
10. Prepare a cell suspension with ~280,000 cells/ml in HEK-Blue™ Detection medium and immediately add 180 µl of the cell suspension 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.

11. 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™ hTLR2-TLR1 cells

As HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1, HEK-Blue™ hTLR2-TLR1 cells will respond to their cognate ligands, such as poly(I:C), flagellin and C_{12}-iE-DAP, respectively.

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ Detection	hb-det2
HEK-Blue™ hTLR2 Cells	hkb-htlr2
HEK-Blue™ hTLR2-TLR6 Cells	hkb-htlr26
HEK-Blue™ hTLR2 KO-TLR1/6 Cells	hkb-htlr2k16
HEK-Blue™ Selection	hb-sel
Pam_2CSK_4 (TLR2-TLR6 ligand)	tlrl-pm2s-1
Pam_3CSK_4 (TLR2-TLR1 ligand)	tlrl-pms

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

TECHNICAL SUPPORT
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