HEK-Blue™ mTLR13 Cells

SEAP Reporter 293 cells expressing the mouse TLR13 gene

Catalog code: hkb-mtlr13

https://www.invivogen.com/hekblue-tlr13

For research use only

Version 22B24-MM

PRODUCT INFORMATION

Contents and Storage

- **3-7 x 10⁶ HEK-Blue™ mTLR13 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 2-8 °C or at -20 °C.*
- **1 ml of Zeocin®** (100 mg/ml). Store at 2-8 °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™ mTLR13 cells should not be passaged more than 20 times to remain fully efficient.

Quality Control

- HEK-Blue™ mTLR13 cells have been stimulated by various pathogen recognition receptor (PRR) agonists. As expected, the TLR13 agonist ORN Sa19 induced the production of SEAP.
- The expression of the mTLR13 gene has been confirmed by RT-PCR.
- 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 13 is an endosomal murine TLR, whose role and ligand remain unclear. Three separate groups have identified 23S ribosomal RNA (rRNA) as a ligand for TLR13[1-3]. This single-stranded rRNA is present in gram-positive and gram-negative bacteria but not in eukaryotic cells. A conserved sequence of 10 residues within the catalytic center of 23S rRNA, "CGGAAAGACC", was found to be both necessary and sufficient to trigger TLR13 signaling. Other forms of rRNA are unable to activate the TLR13 pathway. Thus, unlike other nucleic acid receptors, TLR13 appears to recognize a specific RNA sequence. Interestingly, Oldenburg *et al.* have shown that this sequence is the binding site of the macrolide-lincosamide-streptogramin (MLS) group antibiotics, and that methylation-mediated resistance to these antibiotics (which target A2085 in *S. aureus* 23S rRNA) abolishes the immunostimulatory activity of 23S rRNA[1]. This study suggests that acquisition of antibiotic resistance is a mechanism developed by bacteria to evade host innate immune system. Humans lack TLR13 and probably rely on other pathogen receptors to detect pathogenic bacterial infection. The TLR13 signaling cascade clearly engages MyD88 and UNC93B1, but the details of the pathway require further investigation.

1. Oldenburg M. *et al.*, 2012. TLR13 recognizes bacterial 23S rRNA devoid of erythromycin resistance-forming modification. Science. 337(6098). **2. Hidmark A. *et al.*, 2012.** Cutting edge: TLR13 is a receptor for bacterial RNA. J Immunol. 189(6):2717-21. **3. Li XD & Chen ZJ. 2012.** Sequence specific detection of bacterial 23S ribosomal RNA by TLR13. elife. 1:e00102.

PRODUCT DESCRIPTION

HEK-Blue™ mTLR13 cells are designed for studying the stimulation of mouse TLR13 (mTLR13) by monitoring the activation of NF-κB. HEK-Blue™ mTLR13 cells were obtained by co-transfection of the mTLR13 gene and a secreted embryonic alkaline phosphatase (SEAP) reporter gene 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. Stimulation with a TLR13 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™ mTLR13 cells is HEK-Blue™ Null1-v cells (SEAP reporter cells which do not express mTLR13).

HEK-Blue™ mTLR13 cells are resistant to Blasticidin and Zeocin®.

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 Asia: +852 3-622-34-80
E-mail: info@invivogen.com

SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ mTLR13 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)

- 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 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 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 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™ mTLR13 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™ mTLR13 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 30 µg/ml of Blasticidin and 100 µg/ml of Zeocin®.
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 mTLR13 cells is ~24 hours using the conditions described above.

Cell Handling Recommendations

To ensure the best results:
- Use HEK-Blue™ mTLR13 cells with less than 20 passages.

REPORTER ASSAY

We recommend to use HEK-Blue™ mTLR13 cells with their corresponding parental cell line HEK-Blue™ Null1-v.

Note: For more information regarding the parental cell line please visit https://www.invivogen.com/hek-blue-null1v.

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™ mTLR13 cells (i.e. recombinant human TNF-α), as well as a negative control (such as ORN Sa19 Control, 2 µg/ml).
2. Add 20 µl of a known TLR13 ligand such as ORN Sa19 at 2 µg/ml (final concentration) in a seperate well.

Note: This ligand will induce SEAP activity in HEK-Blue™ mTLR13 cells but not in the parental HEK-Blue™ Null1-v cells.

3. Prepare a suspension of HEK-Blue™ mTLR13 and their parental HEK-Blue™ Null1-v cells by gently rinsing the cells twice with pre-warmed phosphate buffered saline (PBS).
4. Detach the cells by tapping the flask.
5. Transfer the cell suspension to a tube and centrifuge at 300 x g (RCF) for 5 minutes.
6. Discard the supernatant, gently resuspend the cell pellet in pre-warmed PBS and count the cells.
7. Prepare a cell suspension ~220,000 cells per ml in HEK-Blue™ Detection medium and immediately add 180 µl of the cell suspension (~40,000 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.

8. 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™ mTLR13 Cells

As HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1, HEK-Blue™ mTLR13 cells will respond to their cognate ligands, such as poly(I:C), flagellin and C12-iE-DAP, respectively. In order to identify TLR13-specific responses, we recommend to use HEK-Blue™ Null1-v cells as a control cell line.

Note: HEK-Blue™ mTLR13 cells may be stimulated in a TLR13-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
Blasticidin	Selection antibiotic	ant-bl-1
HEK-Blue™ Detection	SEAP detection reagent	hb-det2
HEK-Blue™ Null1-v Cells	Parental cells	hkb-null1v
Normocin™	Antimicrobial reagent	ant-nr-1
ORN Sa19	TLR13 ligand	tlrl-orn19
ORN Sa19 Control	Negative control	tlrl-orn19c
Recombinant human TNF-α	Recombinant cytokine	rcyc-htnfa
Zeocin®	Selection antibiotic	ant-zn-1

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 Asia: +852 3622-3480
E-mail: info@invivogen.com

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

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 Asia: +852 3622-3480
E-mail: info@invivogen.com