

HEK-Blue™ mTLR9 Cells

SEAP Reporter 293 cells expressing the murine TLR9 gene

Catalog code: hkb-mtlr9

<https://www.invivogen.com/hek-blue-mtlr9>

For research use only

Version 23E15-MM

PRODUCT INFORMATION

Contents and storage

- 3-7 x 10⁶ of HEK-Blue™ mTLR9 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 Blastidicin (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™ mTLR9 cells should not be passaged more than 20 times to remain fully functional.

Quality control

- The expression of the murine TLR9 (mTLR9) gene has been confirmed by RT-PCR.
- The expression of mTLR9 has been validated using fluorescence-activated cell sorting (FACS).
- SEAP reporter activity in response to TLR9 agonists has been validated using functional assays.
- The stability for 20 passages following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

PRODUCT DESCRIPTION

HEK-Blue™ mTLR9 cells are designed for studying the murine TLR9 (mTLR9) signaling by monitoring the activation of NF-κB/AP1.

These cells are derived from the human embryonic kidney HEK293 cell line. They express the mTLR9 gene, and a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB and AP-1 binding sites. Levels of SEAP produced upon TLR9 activation can be easily determined in real-time with HEK-Blue™ Detection cell culture medium. HEK-Blue™ Detection offers a one-step colorimetric monitoring of SEAP levels. Alternatively, SEAP activity can be assessed using the alkaline phosphatase detection reagent QUANTI-Blue™ Solution, which allows the same cell cultures to be repeatedly sampled for kinetic studies or further experimentation.

For more information, visit <https://www.invivogen.com/quant-blue>.

HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1.

Note: The parental cell line for HEK-Blue™ mTLR9 cells is HEK-Blue™ Null1 cells (SEAP reporter cells which do not express mTLR9).

BACKGROUND

Toll-Like Receptor 9 (TLR9) is an endosomal receptor that triggers NF-κB- and IRF-mediated pro-inflammatory responses upon the recognition of unmethylated cytosine-phosphorothioate-guanosine (CpG) forms of DNA^{1,3}. Unmethylated CpG dinucleotides are a hallmark of microbial (bacterial, viral, fungal, and parasite) DNA, as well as mitochondrial self-DNA^{3,4}. These TLR9 agonists can be mimicked by synthetic oligonucleotides containing CpG motifs (CpG ODNs), which have been extensively studied to improve adaptive immune responses in the context of vaccination^{1,3}. The structure of the receptor varies by 24% between human TLR9 (hTLR9) and mouse TLR9 (mTLR9)³. They recognize different CpG motifs, the optimal sequences being GTCGTT and GACGTT for hTLR9 and mTLR9, respectively⁵.

1. Kumagai Y. *et al.*, 2008. TLR9 as a key receptor of the recognition of DNA. *Adv. Drug. Deliv. Rev.* 60(7):795-804. 2. Heinz LX. *et al.*, 2021. TASL is the SLC15A4-associated adaptor for IRF5 activation by TLR7-9. *Nature.* 581(7808):316-322. 3. Kayraklioglu N. *et al.*, 2021. CpG oligonucleotides as vaccine adjuvants. *DNA Vaccines: Methods and Protocols. Methods in Molecular Biology.* Vol. 2197, p51-77. 4. Kumar V., 2021. The trinity of cGAS, TLR9, and ALRs: guardians of the cellular galaxy against host-derived self-DNA. *Front. Immunol.* 11:624597. 5. Bauer S. *et al.*, 2001. Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. *Proc Natl Acad Sci USA.* 98(16):9237-42.

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 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.

TECHNICAL SUPPORT

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SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ mTLR9 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, 10% (v/v) fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml Normocin™, 2 mM L-glutamine
- **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 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 preparing 3-4 frozen vials.

2. Aliquot 1 ml of 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™ mTLR9 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 growth medium twice a week.
4. Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

TLR9 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 **ODN 1826**, 100 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™ mTLR9 cells from the incubator and discard growth medium.
 5. Gently rinse cells with pre-warmed PBS (5-10 ml 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™ mTLR9 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: Avoid prolonged incubation of cells at room temperature in **HEK-Blue™ Detection** medium as it may lead to high background or false positive readings.*
9. 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™ mTLR9 Cells

As HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1, HEK-Blue™ mTLR9 Cells will respond to their cognate ligands, such as **poly(I:C)**, **flagellin** and **C12-iE-DAP**, respectively. In order to identify TLR9-specific responses, we recommend to use **HEK-Blue™ Null1** cells as a control cell line.

Note: HEK-Blue™ mTLR9 cells may be stimulated in a TLR9-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 medium	hb-det2
HEK-Blue™ Null1 Cells	Control cell line	hkb-null1
Normocin™	Antimicrobial reagent	ant-nr-1
ODN1826	TLR9 agonist	tlrl-1826
ODN1826 Control	Negative control	tlrl-1826c
QUANTI-Blue™ Solution	SEAP Detection reagent	rep-qbs
Zeocin®	Selection antibiotic	ant-zn-1

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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 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 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 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% 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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