

HEK-Blue™ MD2-CD14 Cells

SEAP Reporter 293 cells expressing the human MD-2 and CD14 genes

Catalog code: hkb-hmdcd

<https://www.invivogen.com/hek-blue-hmd2-cd14>

For research use only

Version 23F05-MM

PRODUCT INFORMATION

Contents and Storage

• 3-7 x 10⁶ of HEK-Blue™ hMD2-CD14 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 Hygromycin B Gold (>90% pure hygromycin B) provided at 100 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 over time that will result in reduced responsiveness 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.

Quality Control

- SEAP reporter activity in response to various pathogen recognition receptor (PRR) agonists has been validated using functional assays. As expected, TLR3 and TLR5 agonists induced the production of SEAP.
- The expression of the human MD-2 and CD14 genes 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 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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Any questions about our cell lines?

Visit our FAQ page.

PRODUCT DESCRIPTION

HEK-Blue™ hMD2-CD14 cells were obtained by co-transfection of the human MD-2/CD14 co-receptor genes and a secreted embryonic alkaline phosphatase (SEAP) reporter gene into HEK293 cells. MD-2 and CD14 act as co-receptors for TLR4 in the detection of bacterial lipopolysaccharide^{1,2}. As HEK-Blue™ hMD2-CD14 cells do not express TLR4, they can be used as control cells for HEK-Blue™ hTLR4 cells (which express the human TLR4 and MD-2/CD14 genes). The SEAP reporter gene in HEK-Blue™ hMD2-CD14 cells is under the control of the IL-12 p40 minimal promoter fused to five NF-κB and AP-1 binding sites.

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™ Solution. With the QUANTI-Blue™ assay, cells are stimulated in a culture medium containing heat-inactivated fetal bovine serum. Following cell activation, QUANTI-Blue™ Solution 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 <https://www.invivogen.com/quant-blue>.

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

Note: The parental cell line for HEK-Blue™ hMD2-CD14 cells is HEK-Blue™ Null2 cells.

1. Shimazu R. *et al.*, 1999. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *J Exp Med*, 189(11): 1777-82.
2. Shuto T. *et al.*, 2005. Membrane-anchored CD14 is required for LPS-induced TLR4 endocytosis in TLR4/MD-2/CD14 overexpressing CHO cells. *BBRC* 338:1402-9.

SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ hMD2-CD14 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.



www.invivogen.com

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% 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 with 20% FBS and 10% (v/v) DMSO

Required Selective Antibiotic(s)

- **Hygromycin B** 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 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™ hMD2-CD14 cells grow as adherent cells. Detach the cells using trypsin for 2-3 min at room temperature (RT).
Note: The response of HEK-Blue™ hMD2-CD14 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 200 µg/ml of **Hygromycin B** 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.

NF-κB 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 **recombinant human TNF-α**, 100 ng/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™ hMD2-CD14 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. Dissociate cell clumps by gently pipetting up and down.
Note: For the reporter assay, do not use trypsin to detach HEK-Blue™ hMD2-CD14 cells.
7. Count cells which have been resuspended in pre-warmed PBS.
Note: For the reporter assay, avoid centrifugation of HEK-Blue™ hMD2-CD14 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: 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.

Notes:

- Strong TLR induction in HEK-Blue™ Detection medium may result in cell cytotoxicity, if this is detrimental to your experiment, we recommend the use of the two-step **QUANTI-Blue™** assay.
- With the **QUANTI-Blue™** assay, stimulate HEK-Blue™ hMD2-CD14 cells in a culture medium containing heat-inactivated fetal bovine serum (FBS; 30 min at 56 °C), as some FBS may contain alkaline phosphatases that can interfere with SEAP quantification.

Specificity of HEK-Blue™ hMD2-CD14 Cells

As HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1, HEK-Blue™ hMD2-CD14 cells will respond to their cognate ligands, such as **poly(I:C)**, **flagellin** and **C12-iE-DAP**, respectively.

RELATED PRODUCTS

Product	Description	Cat. Code
Hygromycin B Gold	Selection antibiotic	ant-hg-1
HEK-Blue™ Detection	SEAP detection medium	hb-det2
HEK-Blue™ Null2 Cells	Parental Cells	hkb-null2
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
Normocin™	Antimicrobial reagent	ant-nr-1
Recombinant hTNF-α	Recombinant Cytokine	rcyc-htrnfα
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

TECHNICAL SUPPORT

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