HEK-Blue™ mMincle Cells

SEAP Reporter 293 cells expressing the murine Mincle gene

Catalog code: hkb-mmcl

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

For research use only

Version 22C17-MM

PRODUCT INFORMATION

Contents and Storage

- **3-7 x 10^6 HEK-Blue™ mMincle 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 puromycin** (10 mg/ml). Store at 4 °C or at -20 °C.*
- **1 ml of blasticidin** (10 mg/ml). Store at 4 °C or at -20 °C.*
- **2 x 1 ml HEK-Blue™ CLR Selection** (250x concentrate); a solution containing several 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™ mMincle cells should not be passaged more than 20 times to remain fully efficient.

Quality Control

- SEAP reporter activity in response to Mincle agonists and various other pathogen recognition receptor (PRR) agonists has been validated using functional assays.
- The cell surface expression of murine Mincle (mMincle) in this cell line has been validated using fluorescence-activated cell sorting (FACS).
- 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.

INTRODUCTION

Mincle is a member of the C-type lectin receptor (CLR) family. Mincle recognizes a variety of exogenous and endogenous stimuli, such as mycobacteria, certain fungi and necrotic cells[1, 2]. Exogenous ligands for Mincle include fungal α-mannose, and the mycobacterial glycolipid, trehalose-6'6'-dimycolate (TDM), also known as cord factor the immunostimulatory component of Mycobacterium tuberculosis[3]. Mincle also binds trehalose-6,6-dibehenate (TDB) which is a synthetic analog of TDM. Furthermore, Mincle senses damaged cells by recognizing the endogenous damage-associated molecular patterns (DAMPs)[4].

Upon ligand recognition Mincle interacts with the Fc receptor common γ-chain (FcRγ), which triggers intracellular signaling through Syk leading to CARD9-dependent NF-κB activation. Syk also induces the mobilization of intracellular calcium (Ca^{2+}) and the activation of the calcineurin-NFAT pathway.

1. Yamasaki S. *et al.*, 2009. C-type lectin Mincle is an activating receptor for pathogenic fungus, Malassezia. PNAS 106(6): 1897–1902. **2. Brown GD. 2008.** Sensing necrosis with Mincle. Nature Immunol. 9:1099-1100. **3. Ishikawa E. *et al.*, 2009.** Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. J Exp Med. 206(13):2879-88. **4. Yamasaki S. *et al.*, 2008.** Mincle is an ITAM-coupled activating receptor that senses damaged cells. Nat Immunol. 9(10):1179-88.

PRODUCT DESCRIPTION

HEK-Blue™ mMincle cells stably express the murine Mincle (mMincle) gene, as well as the genes of the Mincle-NF-κB signaling pathway. They also stably express an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. These reporter cell lines are activated by Mincle ligands, such as trehalose-6,6-dibehenate (TDB) and heat-killed *M. tuberculosis* (HKMT). They do not respond to other CLR ligands such as zymosan, a Dectin-1 and TLR2 ligand.

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™ 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/quanti-blue.

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SAFETY CONSIDERATIONS

Biosafety Level: 2

HEK-Blue™ mMincle 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 Media

- **Growth Medium:** DMEM (4.5 g/l glucose), 10% (v/v) fetal bovine serum (FBS), Pen-Strep (100 U/ml-100 µg/ml), 100 µ/ml Normocin™, 2 mM L-glutamine
- **Freezing Medium:** DMEM, 20% FBS and 10% (v/v) DMSO

Required Selective Antibiotic(s)

- Blasticidin, puromycin and HEK-Blue™ CLR 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 operations 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 1300 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 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.

Frozen Stock Preparation

1. Resuspend cells at a density of 3-7 x 10^6 cells/ml in freezing medium prepared extemporaneously with cold growth medium.

Note: A T-75 culture flask typically yields enough cells to prepare 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™ mMincle 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™ mMincle 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 blasticidin, 1 µg/ml puromycin and 1x HEK-Blue™ CLR Selection.
2. Renew growth medium 2 times a week.
3. Cells should be passaged when a 70-80% confluency is reached. Do not let the cell grow to 100% confluency.

Warning! HEK-Blue™ mMincle cells proliferate more slowly than their parental cell line HEK-Blue™ Null1-v cells. HEK-Blue™ mMincle cells also display a more rounded morphology than their parental cell line.

REPORTER ASSAY

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 96-well plate.
2. Add 20 µl of a positive control (e.g. TDB at 100 µg/ml) in one well.
3. Add 20 µl of a negative control (e.g. sterile endotoxin-free water) in one well.
4. Remove HEK-Blue™-mMincle 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™ mMincle cells.

7. Count cells which have been resuspended in pre-warmed PBS.

Note: For the reporter assay, avoid centrifugation of HEK-Blue™ mMincle 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% 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™ mMincle cells

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

RELATED PRODUCTS

Product	Description	Cat. Code
Anti-mMincle-IgG	Mincle antibody	mabg-mmcl
Blasticidin	Selection antibiotic	ant-bl-1
HKMT	Heat-killed *M. tuberculosis*	tlrl-hkmt-1
HEK-Blue™ CLR Selection	Selection antibiotics	hb-csm
HEK-Blue™ Detection	SEAP detection medium	hb-det2
HEK-Blue™ Null1-v cells	Parental cell line	hkb-null1v
Puromycin	Selection antibiotic	ant-pr-1
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
TDB	Trehalose-6,6-dibehenate	tlrl-tdb

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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 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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