HEK-Blue™ Null2-k Cells

SEAP reporter 293 parental cell line
Catalog code: hkb-null2-k
https://www.invivogen.com/hek-blue-null2-k

For research use only
Version 23E15-MM

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10^6 HEK-Blue™ Null2-k 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 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 resulting 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

- Activation of the NF-κB response has been verified upon stimulation with various inducers.
- 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.

PRODUCT DESCRIPTION

HEK-Blue™ Null2-k cells derive from human embryonic kidney 293 (HEK293) cells. HEK-Blue™ Null2-k cells express a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of the IL-12 p40 minimal promoter to five NF-κB and AP-1 binding sites. Stimulation of HEK-Blue™ Null2-k cells with an NF-κB and/or AP-1 activator induces the production of SEAP. These cells express endogenous levels of TLR3, TLR5, NOD1.

The HEK-Blue™ Null2-k cell line is the parental cell line of:

- **Overexpressing TLR cell lines:** HEK-Blue™ mTLR7.

Note: For more information regarding these cell lines, please visit https://www.invivogen.com/hek-blue-mtlr7.

Levels of SEAP produced by HEK-Blue™ Null2-k cells can be easily determined using HEK-Blue™ Detection: a cell culture medium that contains all the required nutrients for cell growth, as well as a specific SEAP color substrate that allows for real-time detection of SEAP activity. The hydrolysis of the color substrate by SEAP produces a purple/blue color that can be easily detected with the naked eye or quantitatively measured with a spectrophotometer (OD620-655 nm). HEK-Blue™ Detection can be used in high-throughput screening (HTS).

In both detection methods, the hydrolysis of the substrate by SEAP produces a purple/blue color that can be easily detected with the naked eye or quantitatively measured with a spectrophotometer (OD 620-655 nm). Both methods are applicable to high-throughput screening (HTS).

SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ Null2-k 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.

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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), 100 µg/ml Normocin™, Pen-Strep (100 U/ml-100 µg/ml)
- **Freezing Medium:** DMEM, 4.5 g/l glucose, 20% FBS, 10% DMSO

Required Selection Antibiotics

- 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 (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 larger tube containing 15 ml of pre-warmed growth medium. **Do not add Zeocin® 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.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 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 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. HEK-Blue™ Null2-k 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 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.

Cell Handling Recommendations

To ensure the best results:
- Use HEK-Blue™ Null2-k cells with less than 20 passages.

REPORTER ASSAY

Below is a protocol for using InvivoGen's HEK-Blue™ Null2-k cells for studying the activation of NF-κB and/or AP-1. Expression levels of SEAP can be readily assessed using either HEK-Blue™ Detection as outlined below.

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 (i.e. recombinant human TNF-α) in one well.
3. Add 20 µl of a negative control (such as sterile water) in one well.
4. Remove HEK-Blue™ Null2-k Cells from the incubator and discard growth medium.
5. Gently rinse the cells twice with pre-warmed phosphate buffered saline (PBS).
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. Transfer the cell suspension to a tube and centrifuge at 150 x g (RCF) for 10 minutes or 300 x g (RCF) for 5 minutes.
8. Discard the supernatant, gently resuspend the cell pellet in pre-warmed PBS and count the cells
9. Prepare a cell suspension of ~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.

10. Incubate the plate at 37 °C in a CO_2 incubator for 16-24h. SEAP detection can be observed with the naked eye and accurately determined using a spectrophotometer at 620-655 nm.

Alternatively, SEAP can be detected using **QUANTI-Blue™ Solution**, a convenient and highly sensitive reagent that allows for repeat sampling or further experimentation. For more information please visit our website: https://www.invivogen.com/quanti-blue.

RELATED PRODUCTS

Product	Description	Cat. Code
HEK-Blue™ Detection	SEAP detection medium	hb-det2
Normocin™	Antimicrobial reagent	ant-nr-1
Zeocin®	Selective antibiotic	ant-zn-1

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

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