HEK-Blue™ KO-TIFA Cells

TIFA knockout SEAP reporter cells

Catalog code: hkb-kotifa

https://www.invivogen.com/ko-alpk1-tifa-cells

For research use only

Version 19L23-ED

PRODUCT INFORMATION

Contents and Storage

- **3-7 x 10^6 HEK-Blue™ KO-TIFA 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. HEK-Blue™ KO-TIFA cells should not be passaged more than 20 times to remain fully functional.

Quality Control

- *TIFA* gene knockout has been verified by DNA sequencing, RT-qPCR, and functional assays.
- The stability for 20 passages following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

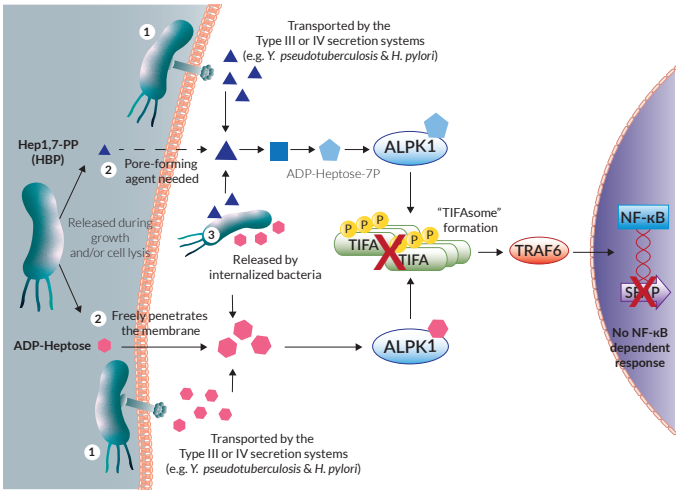
CELL LINE DESCRIPTION

HEK-Blue™ KO-TIFA cells were generated from HEK-Blue™ Null1-v cells through the stable knockout of the *TIFA* gene. The parental cells derive from human embryonic kidney 293 (HEK-293) cells. HEK-Blue™ KO-TIFA cells express a secreted embryonic alkaline phosphatase (SEAP) under the control of an NF-κB-inducible promoter comprised of an IFN-β minimal promoter fused to five NF-κB and AP-1 binding sites. Levels of SEAP in the supernatant can be easily determined with HEK-Blue™ Detection, a SEAP detection cell culture medium. Unlike their parental cell line, HEK-Blue™ KO-TIFA cells do not respond to cytosolic ADP-Heptose. However, they do respond to other NF-κB inducers such as TNF-α and IL-1β. HEK-Blue™ KO-TIFA cells are resistant to Zeocin™

BACKGROUND

TRAF interacting forkhead-associated protein A (TIFA) is an important mediator in the cytoplasmic surveillance pathway of pathogenic Gram-negative bacteria such as *Shigella flexneri* and *Helicobacter pylori*[1,2]. Activation of the TIFA signaling pathway is triggered by ADP-β-d-manno-heptose (ADP-Heptose) and d-glycero-β-d-mannoheptose-1,7-bisphosphate (HBP), metabolic intermediary sugars in the biosynthesis of lipopolysaccharide (LPS), an essential component of the outer membrane of Gram-negative bacteria. Upon entering the cytosol, these bacterial sugars interact with and activate a central cytosol sensor receptor, Alpha protein kinase 1 (ALPK1)[3]. Activated ALPK1 then phosphorylates the N-terminal T9 domain of TIFA, inducing its oligomerization and triggering the formation of 'TIFAsomes'. This activates the E3 ubiquitin ligase TRAF6, and ultimately results in the initiation of an NF-κB dependent pro-inflammatory response, releasing cytokines such as interleukin (IL)-8[3,4]. Interestingly, transcrptional regulation of the TIFA-NF-κB pathway has been shown to be crucial in the endothelial innate immune response by potentiating and amplifying the NLRP3 inflammasome response[5].

Targeting this pathway will be important in the development of novel treatments for modulating inflammation caused by bacterial infection as well as pathologies associated with its dysregulation such as vascular diseases and gastric cancer.



1. Gall, A. *et al.* 2017. TIFA Signaling in Gastric Epithelial Cells Initiates the cag Type 4 Secretion System-Dependent Innate Immune Response to *Helicobacter* pylori Infection. MBio 8. **2. Garcia-Weber, D. *et al.* 2018.** ADP-heptose is a newly identified pathogen-associated molecular pattern of Shigella flexneri. EMBO Rep 19. **3. Zhou, P. *et al.* 2018.** Alpha-kinase 1 is a cytosolic innate immune receptor for bacterial ADP-heptose. Nature 561, 122-126. **4. Milivojevic, M. *et al.* 2017.** ALPK1 controls TIFA/TRAF6-dependent innate immunity against heptose-1,7-bisphosphate of gram-negative bacteria. PLoS Pathog 13, e1006224.. **5. Lin, T. *et al*, 2016.** TIFA as a crucial mediator for NLRP3 inflammasome. PNAS 113, 15078-15083.

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 Hong Kong: +852 3622-3480

E-mail: info@invivogen.com

InvivoGen

www.invivogen.com

SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ KO-TIFA 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, 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

Note: Some FBS may contain alkaline phosphatases that can interfere with SEAP quantification. We recommend to use heat-inactivated FBS to inactivate these thermosensitive enzymes.

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 1000-1200 RPM (200-300 x *g*) 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 5-7x 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. Maintain and subculture the cells in growth medium supplemented with 100 µg/ml of Zeocin™.
2. Renew growth medium twice a week.
3. 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™ KO-TIFA cells with less than 20 passages.

REPORTER ASSAY

We recommend to use HEK-Blue™ KO-TIFA 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™ KO-ALPK1 cells (i.e. recombinant human TNF-α), as well as a negative control (i.e. culture medium only).
2. Add 20 µl of a known ALPK1 ligand such as ADP-Heptose at 0.3 µg/ml (final concentration) in a seperate well.

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

3. Prepare a suspension of HEK-Blue™ KO-TIFA 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 using trypsin or by tapping the flask.
5. Transfer the cell suspension to a tube and centrifuge at 200-300 x *g* for 5 minutes.
6. Discard the supernatant, gently resuspend the cell pellet in pre-warmed PBS and count the cells
7. Prepare cell suspensions of ~280,000 cells per ml in HEK-Blue™ Detection medium and immediately add 180 µl of the cell suspensions (~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.

8. 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

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.

RELATED PRODUCTS

Product	Cat. Code
HEK-Blue™ Null1-v cells	hkb-null1v
HEK-Blue™ KO-ALPK1 cells	hkb-koalpk1
ADP-Heptose	tlrl-adph
Recombinant human TNF-α	rcyc-htnfa
HEK-Blue™ Detection	hb-det2
QUANTI-Blue™ Solution	rep-qbs

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 Hong Kong: +852 3-622-34-80
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 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
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