HEK-Blue™ TGF-β Cells

Tumor growth factor beta reporter cells

Catalog code: hkb-tgfbv2

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

For research use only

Version 21J11-NJ

PRODUCT INFORMATION

Contents:

- **1 vial of HEK-Blue™ TGF-β cells** (3-7 x 10^5 cells) in freezing medium

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). Normocin™ is 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 ml of QB reagent** and **1 ml of QB buffer** (sufficient to prepare 100 ml of **QUANTI-Blue™ Solution**, a SEAP detection reagent). QB reagent and QB buffer are stable for 1 year at -20 °C. QUANTI-Blue™ Solution is stable for 2 weeks at 4 °C and for 2 months at -20 °C.

Note: Data sheets for all components are available on our website.

Handling Cells Upon Receipt

Cells must be thawed **immediately** upon receipt and grown according to handling procedures (as described on the next page), to ensure 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™ TGF-β cells should not be passaged more than 20 times to remain fully efficient.

Quality Control

- SEAP reporter activity in response to human and murine TGF-β has been validated using functional assays.
- The stability for 20 passages following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

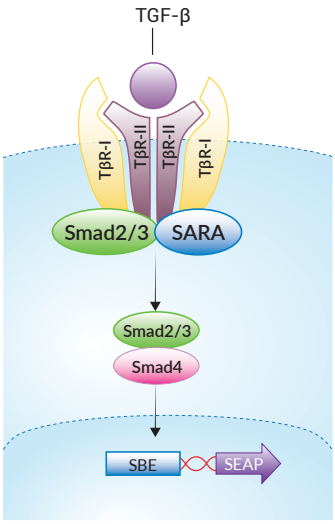
USE RESTRICTIONS

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.

BACKGROUND

Tumor growth factor-beta (TGF-β) belongs to a family of structurally related cytokine that regulate a plethora of cellular functions, such as proliferation, apoptosis, differentiation and migration[1, 2]. TGF-β binds to type II receptors (TβR-II) which recruit and activate type I receptors (TβR-I). The active ligand-heterotetrameric receptor complex signals through downstream transcriptional factors named Smads, including Smad2, Smad3, and Smad4. Smad complexes translocate into the nucleus where they regulate the transcription of target genes, which contain one or more Smad binding elements (SBEs) in their promoter region[3]. Perturbations in TGF-β signaling affect immune homeostasis and tolerance, leading to inflammatory diseases and tumor immune evasion[3].



1. Travis M.A. & Sheppard D., 2014. TGF-B activation and function in immunity. Annu. Rev. Immunol. 32:51. 2. Taylor A.W., 2009. Review of the activation of TGF-beta in immunity. J. Leukoc. Biol. 85(1):29. 3. Battle E. & Massagué J., 2019. Transforming Growth Factor-beta Signaling in Immunity and Cancer. Immunity. 50(4):924.

PRODUCT DESCRIPTION

HEK-Blue™ TGF-β cells were engineered from the human embryonic kidney HEK 293 cell line to detect bioactive human and murine TGF-β by monitoring the activation of the TGF-β/Smad pathway. These cells can also be used for screening anti-TGF-β antibodies using flow cytometry. HEK-Blue™ TGF-β cells were generated by stable overexpression of a Smad-inducible secreted embryonic alkaline phosphatase (SEAP) reporter. Binding of TGF-β to its receptor on the surface of HEK-Blue™ TGF-β cells triggers a signaling cascade leading to the formation of a Smad3/Smad4 complex. The heterocomplex enters the nucleus and binds SBE sites inducing the production of SEAP. This can be readily assessed using QUANTI-Blue™ Solution. HEK-Blue™ TGF-β cells are resistant to Zeocin™.

Detection range for human TGF-β: 10 pg/ml - 10 ng/ml
Detection range for murine TGF-β: 10 pg/ml - 10 ng/ml

TECHNICAL SUPPORT
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FAQ Any questions about our cell lines? Visit our FAQ page.

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www.invivogen.com

SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ TGF-β 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% (v/v) 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, 20% (v/v) FBS, 10% (v/v) DMSO
- **Test Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% (v/v) heat-inactivated FBS, Pen-Strep (100 U/ml - 100 µg/ml), **without Zeocin™ and Normocin™**

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 Selective Antibiotic(s)

- 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 selection antibiotics until the cells have been passaged twice.**
4. Centrifuge vial at 300 x g (RCF) for 5 mins.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium without selection antibiotics.
6. Transfer the vial contents to a T-25 culture flask containing 5 ml of growth medium.

Note: To avoid excessive alkalinity of the medium during recovery of the cells, place the tissue culture flask containing the growth medium into the incubator for at least 15 minutes prior to the addition of the vial contents.

7. Place the culture at 37 °C in 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 3-5 x 10^6 cells/ml in freshly prepared freezing medium with cold DMEM.

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™ TGF-β cells grow as adherent cells. Detach the cells in the presence of phosphate buffered saline (PBS) by tapping the flask or by using a cell scraper.
2. Maintain and subculture the cells in growth medium supplemented with 100 µg/ml 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.

Note: The average doubling time for the HEK-Blue™ TGF-β cells is ~24 hours using the conditions described above.

DETECTION OF TGF-β

Day 1

1. Prepare HEK-Blue™ TGF-β cell suspension: gently rinse cells twice with pre-warmed PBS, detach the cells in the presence of PBS by tapping the flask, resuspend cells in fresh, pre-warmed test medium (containing heat-inactivated FBS) and prepare a cell suspension at 2.77 x 10^5 cells/ml.

Note: The response of HEK-Blue™ TGF-β cells can be altered by the action of trypsin. Do not use trypsin to detach HEK-Blue™ TGF-β cells.

2. Add 20 µl of sample per well of a flat-bottom 96-well plate.
3. In separate wells, add 20 µl of a positive control, such as recombinant human TGF-β (final concentration 1 ng/ml), and 20 µl of a negative control, such as recombinant human TNF-α (final concentration 10 ng/ml).
4. Add 180 µl of HEK-Blue™ TGF-β cell suspension (~5 x 10^4 cells) per well.
5. Incubate overnight at 37 °C in 5% CO_2.

Day 2

6. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed product data sheet.
7. Add 20 µl of induced HEK-Blue™ TGF-β cell supernatant.
8. Add 180 µl of resuspended QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
9. Incubate the plate at 37 °C for 30 min to 3 hours.
10. Determine SEAP levels using a spectrophotometer at 620-655 nm.

Alternatively, the SEAP activity in HEK-Blue™ TGFβ cell supernatant can be detected using InvivoGen's HEK-Blue™ Detection, a cell culture medium that allows real-time detection of SEAP activity.

RELATED PRODUCTS

Product	Description	Cat. Code
Zeocin™	Selection antibiotic	ant-zn-1
Normocin™	Antimicrobial reagent	ant-nr-1
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs
HEK-Blue™ Detection	SEAP detection medium	hb-det2
Recombinant human TGF-β1	Recombinant cytokine	rcyc-htgfb1

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