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 24K16-NJ

PRODUCT INFORMATION

Contents:

- **3-7 x 10^6 of HEK-Blue™ TGF-β cells** n a cryovial or shipping flask. *Note: 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 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 Frozen Cells Upon Arrival

Cells are shipped in dry ice, and upon receipt should immediately be thawed for culture or stored below -130°C, preferably in liquid nitrogen vapor, for long-term storage.

IMPORTANT: Do not store cell vials at -80°C as this will decrease cell viability and performance. Contact technical support if the cells are not frozen or in dry ice upon arrival.

To insure the highest level of viability and best assay performance, we strongly recommend that you thaw the cells and initiate the culture as soon as possible upon receipt (as described on the next page).

Warranties

- InvivoGen's cells are provided 'AS IS' and their viability is guaranteed upon shipment from our facilities for a period of 30 days, provided that the customer has properly stored and handled the product.
- Our cell lines are guaranteed free of mycoplasma contamination.
- The stability of our cell lines is guaranteed for 20 passages.

Quality Control

- SEAP reporter activity in response to TGF-β is validated using functional assays.
- The stability for 20 passages following thawing is confirmed.
- These cells are tested for mycoplasma contamination.

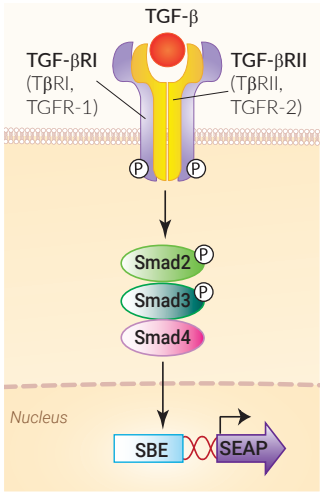
USE RESTRICTIONS

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 outlicensing@invivogen.com.

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. HEK-Blue™ TGF-β cells were generated by stable transfection 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 (Smad binding elements) sites in the engineered minimal promoter, thereby 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

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.

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

InvivoGen
www.invivogen.com

SAFETY CONSIDERATIONS

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 (HI) 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) HI FBS, Pen-Strep (100 U/ml-100 µg/ml) **without Normocin™ and Zeocin®**

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. ***Warning: Do not add selection antibiotics until the cells have been passaged twice.***
4. Centrifuge vial at 150 x g (RCF) for 10 minutes.
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 25 cm^2 tissue culture flask containing 5 ml of growth medium without selection antibiotics.

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 5-7 x 10^6 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. Prepare 1 ml aliquots of cells in 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 Handling Recommendations

To ensure the best results, use HEK-Blue™ TGF-β cells with less than 20 passages.

Cell Maintenance

1. HEK-Blue™ TGF-β cells grow as adherent cells. Detach the cells using PBS at 37°C or trypsin at room temperature (RT) for 2-3 min. ***Warning: Prolonged action of trypsin or incubation at 37°C may alter the cell surface expression of cytokine receptors.***
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.

DETECTION OF TGF-β ACTIVITY

We recommend to use **test medium** one passage prior to the assay.

Day 1:

1. Prepare HEK-Blue™ TGF-β cell suspension: gently rinse cells twice with pre-warmed phosphate buffered saline (PBS), detach the cells using PBS at 37°C or trypsin at room temperature (RT) for 2-3 min. Tap the flask if needed. Resuspend cells in fresh, pre-warmed test medium and prepare a cell suspension at ~280,000 cells/ml.
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 (1 ng/ml final concentration), and 20 µl of a negative control, such as recombinant human TNF-α (10 ng/ml final concentration).
4. Add 180 µl of HEK-Blue™ TGF-β cell suspension (~50,000 cells) per well.
5. Incubate overnight at 37 °C in 5% CO_2.

Day 2:

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

RELATED PRODUCTS

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

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