HEK-Blue™ TGF-β Cells
Tumor growth factor beta reporter cells
Catalog code: hkb-tgfb
https://www.invivogen.com/hek-blue-tgfb
For research use only
Version 19I04-MM

PRODUCT INFORMATION

Contents
- 1 vial of HEK-Blue™ TGF-β cells (3-7 x 10^6 cells)
- 1 ml of Normocin™ (50 mg/ml), a formulation of three antibiotics active against mycoplasmas, bacteria, and fungi. Store at -20°C.*
- 1 ml of Blasticidin (10 mg/ml). Store at 4°C or at -20°C.*
- 1 ml of Hygromycin B Gold (>90% purity; 100 mg/ml). Store at 4°C or at -20°C.*
- 1 ml of Zeocin™ (100 mg/ml). Store at 4°C or at -20°C.*
- 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.

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.

Handling 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.

Cell Line Stability
Cells will undergo genotypic changes over time that will result 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
- Expression of TGFBRI, Smad3 and Smad4 has been confirmed by RT-PCR.
- SEAP reporter activity in response to TGF-β and various cytokines 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.

INTRODUCTION

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.

TGF-β binds to a type II receptor which recruits and activates a type I receptor. The type I receptor then phosphorylates receptor-regulated Smads (R-Smads), such as Smad2 and Smad3, which associate with Smad4.

R-Smad/Smad4 complexes then accumulate in the nucleus where they regulate the transcription of target genes.

CELL LINE DESCRIPTION

HEK-Blue™ TGF-β cells allow the detection of bioactive TGF-β by monitoring the activation of the TGF-β/Smad pathway. They were generated by stable transfection of HEK293 cells with the human TGFBRI, Smad3 and Smad4 genes. They further express a SEAP reporter gene under the control of the β-globin minimal promoter fused to three Smad3/4-binding elements (SBE).

Stimulation of HEK-Blue™ TGF-β cells with TGF-β induces the activation of the TGF-β/Smad signaling pathway leading to the formation of a Smad3/Smad4 complex. The heterocomplex enters the nucleus and binds SBE sites inducing the production of SEAP. The quantity of SEAP secreted in the supernatant can be easily assessed using QUANTI-Blue™ Solution, a SEAP detection reagent.

TGF-β-mediated SEAP production can be blocked using a neutralizing antibody, such as anti-hTGF-β.

HEK-Blue™ TGF-β cells are resistant to blasticidin, hygromycin B, and Zeocin™.

Detection range for human TGF-β: 0.1 - 10 ng/ml.
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 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
- Test Medium: DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% heat-inactivated FBS, Pen-Strep (100 U/ml-100 μg/ml) without Normocin™, Blasticidin, Hygromycin B, and 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 to a larger tube containing 15 ml of pre-warmed growth medium. Do not add Blasticidin, Hygromycin B, and Zeocin™ until the cells have been passaged twice.
4. Centrifuge at 1000-1200 RPM (RCF 200-300 g) for 5 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium without selection antibiotics.
6. Transfer the cells to a T-25 culture flask containing 5 ml of growth medium.
7. Place the culture at 37 °C in 5% CO2.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10^6 cells/ml in freezing medium freshly prepared with cold DMEM.

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 maintenance

- Maintain and subculture the cells in growth medium supplemented with 30 μg/ml of blasticidin, 200 μg/ml of Hygromycin B Gold and 100 μg/ml of Zeocin™.
- Renew growth medium twice a week.
- Cells should be passaged when a 70-80% confluency is reached. Do not let the cell grow to 100% confluency.

Cell Handling Recommendations

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

REPORTER ASSAY

Day 1:

1. Prepare HEK-Blue™ TGF-β cell suspension: gently rinse cells twice with pre-warmed phosphate buffered saline (PBS) and then detach the cells in the presence of PBS by tapping the flask. Resuspend cells in fresh, pre-warmed test medium and prepare a cell suspension at ~280,000 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 each sample per well of a flat-bottom 96-well plate.
3. Add 20 μl of recombinant human TGF-β at 10 ng/ml (positive control) in one well.
4. Add 20 μl of a recombinant cytokine such as recombinant human IL-4 at 10 ng/ml (negative control) in one well.
5. Add 180 μl of cell suspension (~50,000 cells) per well.
6. Incubate the plate at 37 °C in a CO2 incubator for 20-24 h.

Day 2:

1. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed data sheet.
2. Add 20 μl of induced HEK-Blue™ TGF-β cell 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 1-3 h.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Cat. Code</th>
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<td>Blasticidin</td>
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<td>Hygromycin B Gold</td>
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<td>Normocin™</td>
<td>Antimicrobial Reagent</td>
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<td>QUANTI-Blue™ Solution</td>
<td>SEAP detection reagent</td>
<td>rep-qbs</td>
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<td>Recombinant human IL-4</td>
<td>Recombinant cytokine</td>
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<td>Recombinant human TGF-β</td>
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<tr>
<td>Zeocin™</td>
<td>Selective antibiotic</td>
<td>ant-zn-1</td>
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QUANTI-Blue™ Solution
Medium for detection and quantification of alkaline phosphatase in standard and HTS assays
Catalog code: rep-qbs, rep-qbs2
https://www.invivogen.com/quanti-blue

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Version 19F11-MM

PRODUCT INFORMATION

Contents
QUANTI-Blue™ Solution is available in two pack sizes:
- rep-qbs containing 5 x 1 ml of QB reagent and 5 x 1 ml QB buffer to prepare 500 ml of QUANTI-Blue™ Solution sufficient for 25 x 96-well plates (standard procedure) or 20 x 1536-well plates (HTS screening)
- rep-qbs2 containing 10 x 1 ml of QB reagent and 10 x 1 ml QB buffer to prepare 1 liter of QUANTI-Blue™ Solution sufficient for 50 x 96-well plates (standard procedure) or 40 x 1536-well plates (HTS screening)

Required Material (not provided)
- Sterile water
- Sterile screw cap tube, glass bottle or flask

Storage and Stability
- Store QB reagent and QB buffer at -20°C. Product is stable for 1 year at -20°C when properly stored.
- Reconstituted QUANTI-Blue™ Solution is stable for 2 weeks at 2-8°C and for 2 months at -20°C. Protect QUANTI-Blue™ from light.

Quality Control
Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.
- Physicochemical characterization (including pH, solubility).
- Functional assays using alkaline phosphatase or SEAP-expressing reporter cells.

DESCRIPTION
QUANTI-Blue™ is a colorimetric enzyme assay developed to determine any alkaline phosphatase activity (AP) in a biological sample, such as supernatants of cell cultures. QUANTI-Blue™ Solution changes from pink to a purple-blue color in the presence of AP. Secreted embryonic alkaline phosphatase (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 is used as a reporter gene. It is a truncated form of placental alkaline phosphatase, a GPI-anchored protein. SEAP is secreted into cell culture supernatant and is used as a reporter gene.

FEATURES AND ADVANTAGES
- Requires small samples of cell supernatants - 20 µl is sufficient.
- No need to process samples - Preparation of cell lysates or heating of samples is not required.
- Determine secreted AP activity without disturbing cells - The same cell cultures can be repeatedly sampled for kinetic studies.
- Assay can be completed in 30 min - Hands-on time no longer than 10 min. The enzymatic activity can be detected as early as 15 min after incubation of the samples in QUANTI-Blue™ Solution.
- Wide dynamic range allows to detect low and high levels of AP - No need to perform multiple sample dilutions.
- Highly sensitive for quantitative measurement - Higher saturation threshold than with pNPP (p-nitrophenyl phosphate) resulting in more significant differences between no, low or high AP activity.
- Extremely simple to use - 1) Prepare solution with water, 2) add sample to detection reagent, 3) incubate at 37°C, and 4) assess AP activity.

METHODS
QUANTI-Blue™ Solution has been optimized for use in 96-well plates (standard procedure) and in 1536-well plates (high throughput screening procedure).

A. Standard procedure

1. Prepare 100 ml of QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml QB buffer to 98 ml of sterile water in a sterile glass bottle or flask.
2. Mix well by vortexing and incubate at room temperature for 10 min before use.
3. Use QUANTI-Blue™ Solution immediately or store at 2-8°C or -20°C.
4. Add 1 ml QB reagent and 1 ml QB buffer to 98 ml sterile H₂O.
5. Add 20 µl of sample (supernatant of SEAP-expressing cells) or supernatant to 96-well plate.
6. Incubate at 37°C for 15 min to 6 h.
7. Measure optical density (OD) at 620-655 nm using a microplate reader.

For different cell culture plate formats, please refer to the table below:

<table>
<thead>
<tr>
<th></th>
<th>96-well plate</th>
<th>24-well plate</th>
<th>12-well plate</th>
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<tbody>
<tr>
<td>QUANTI-Blue™</td>
<td>180 µl</td>
<td>450 µl</td>
<td>900 µl</td>
</tr>
<tr>
<td>Supernatant</td>
<td>20 µl</td>
<td>50 µl</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

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TECHNICAL SUPPORT
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www.invivogen.com
B. High Throughput Screening (HTS) procedure

This procedure has been optimized for use in HTS screening procedures in 1536-well plates. QUANTI-Blue™ Solution is added directly to the cell suspension to reduce liquid handling. Ensure QB reagent and QB buffer are completely thawed before use. 

1. Dispense cell suspension and test compounds into a 1536-well plate in a volume that does not exceed 5 µl per well. Incubate cells with test compounds for the desired period of time.
2. Prepare 17 ml of QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml of QB buffer to 15 ml of sterile water in a 50 ml screw cap tube.
3. Mix well by vortexing and incubate at room temperature for 10 minutes before use.
4. Use QUANTI-Blue™ Solution immediately or store at 2-8°C or -20°C.
5. Dispense 2 µl of QUANTI-Blue™ Solution to the wells containing ≤5 µl of cell culture in a 1536-well plate.
6. Mix using a plate shaker.
7. Incubate at 37°C for 15 min to 6 h.
8. Measure OD at 620-655 nm.

**Note:** If the negative control turns purple/blue, it means the fetal bovine serum (FBS) contains alkaline phosphatase. We recommend to heat FBS at 56°C for 30 min to inactivate the alkaline phosphatase activity.

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**RELATED PRODUCTS**

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<th>Product</th>
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<td>pNiFty2-SEAP (Zeo⁺)</td>
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<td>pSELECT-zeo-SEAP</td>
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<tr>
<td>HEK-Blue™ Detection</td>
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<tr>
<td>Recombinant SEAP Protein</td>
<td>rec-hseap</td>
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</tbody>
</table>

**Reporter cells**

- HEK-Blue™ hTLR2
- HEK-Blue™ hTLR4
- RAW-Blue™ Cells
- THP1-Blue™ NF-κB Cells
- THP1-Blue™ ISG Cells

For a complete list of InvivoGen's Reporter Cell Lines visit [https://www.invivogen.com/reporter-cells](https://www.invivogen.com/reporter-cells)

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**Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.**