HEK-Blue™ TSLP Cells

Thymic stromal lymphopoietin reporter cells

Catalog code: hkb-tslp

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

For research use only

Version 24H26-NJ

PRODUCT INFORMATION

Contents

- **3-7 x 10^6 of HEK-Blue™ TSLP cells** in a cryovial or shipping flask. *Note: If cells provided in a cryovial are not frozen upon arrival, contact InvivoGen immediately.*
- **2 x 1 ml of HEK-Blue™ Selection** (250X concentrate), a solution containing several selection antibiotics. 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.

IMPORTANT: This cell line requires special maintenance as described in the following protocol.

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

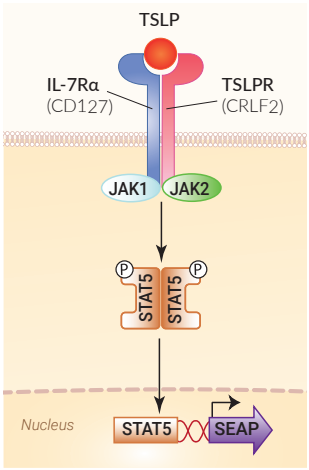
- SEAP reporter activity in response to TSLP is validated using functional assays.
- The stability for 20 passages following thawing is confirmed.
- These cells are tested for mycoplasma contamination.

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™ TSLP reporter cells have been specifically designed to detect bioactive human (h) thymic stromal lymphopoietin (TSLP) by monitoring the activation of the JAK/STAT pathway.

HEK-Blue™ TSLP cells were generated by stable transfection of the human embryonic kidney HEK293 cell line with the genes encoding human TSLPR, IL-7Rα, and STAT5 genes, to obtain a fully active TSLP signaling pathway. In addition, a STAT5-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene was also introduced. Upon TSLP stimulation, HEK-Blue™ TSLP cells trigger the JAK/STAT5 activation and the subsequent secretion of SEAP, which can be readily monitored using QUANTI-Blue™ Solution, a SEAP detection medium. HEK-Blue™ TSLP cells detect human but not murine TSLP. Of note, these cells also respond, to a weaker extent, to human IFN-γ. However, they do not respond to other STAT5-signaling cytokines: IL-2, IL-7, IL-9, and IL-15.

HEK-Blue™ TSLP cells are resistant to Blasticidin, Hygromycin B, and Zeocin®.

Detection range for human TSLP: 300 pg/ml - 100 ng/ml

No detection for mouse TSLP

BACKGROUND

Thymic stromal lymphopoietin (TSLP) is a cytokine produced by epithelial and stromal cells in barrier tissues as well as innate immune cells such as dendritic cells, basophils and mast cells. It has pleiotropic actions on a wide range of immune and non-immune cell types[1]. TSLP belongs to the common γ chain (γc) cytokine family. Its receptor comprises the IL-7Rα (CD127) and TSLPR (CRLF2), a γc-like subunit. It signals through tyrosine kinases of the Janus family (JAK1 and JAK2) and signal transducer and transcription activators (STATs), notably STAT5[1]. TSLP is a pleiotropic cytokine best known as a critical mediator of type 2 immune responses and a promoter of Th2 cell-mediated diseases (e.g. asthma)[1,2]. Emerging evidence indicates that TSLP is also implicated in viral infections, cancer, chronic inflammation and fat metabolism[1].

1. Ebina-Shibuya R. and Leonard WJ., 2023. Role of thymic stromal lymphopoietin in allergy and beyond. Nat Rev Immunol, 23(1):24-37. **2. Schmitt P. *et al.*, 2024.** TL1A is an epithelial alarmin that cooperates with IL-33 for initiation of allergic airway inflammation. J Exp Med. 221(6) e20231236.

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

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www.invivogen.com

SAFETY CONSIDERATIONS

HEK-Blue™ TSLP 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 HEK-Blue™ Selection**

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)

- HEK-Blue™ Selection

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 75 cm^2 tissue culture flask containing 20 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™ TSLP cells with less than 20 passages.

Note: These cells detach easily. Please handle with care.

Cell Maintenance

1. HEK-Blue™ TSLP 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 1X HEK-Blue™ Selection.
3. Examine the cell culture every weekday. Renew growth media regularly and add extra volume over the weekend.
4. Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

DETECTION OF TSLP ACTIVITY

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

Day 1:

1. Prepare HEK-Blue™ TSLP 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 TSLP (10 ng/ml final concentration), and 20 µl of a negative control, such as recombinant human IFN-α (1000 U/ml final concentration).
4. Add 180 µl of HEK-Blue™ TSLP 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™ TSLP 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
HEK-Blue™ Selection	Selection antibiotic mix	hb-sel
Normocin™	Antimicrobial reagent	ant-nr-1
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
Recombinant human IFN-α2b	Recombinant cytokine	rcyc-hifna2b

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