

HEK-Blue™ IL-11 Cells

Interleukin-11 reporter cells

Catalog code: hkb-hil11r

<https://www.invivogen.com/hek-blue-il11>

For research use only

Version 21J11-NJ

PRODUCT INFORMATION

Contents

- 3-7 x 10⁶ HEK-Blue™ IL-11 cells in a cryovial or shipping flask.

IMPORTANT: 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. HEK-Blue™ Selection can be stored 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 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 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™ IL-11 cells should not be passaged more than 20 times to remain fully efficient.

Quality Control

- The expression of human IL-11R α has been confirmed by RT-qPCR.
- SEAP reporter activity in response to human and murine IL-11 has been validated using functional assays.
- The stability for 20 passages following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

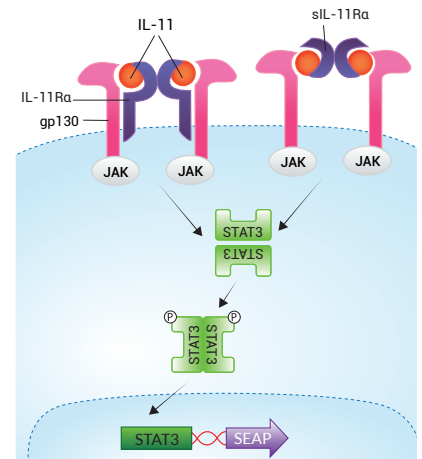
USE RESTRICTIONS

These cells are distributed for research purposes only.

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BACKGROUND

The cytokine interleukin 11 (IL-11) belongs to the IL-6 family^{1, 2}. IL-11 has pleiotropic physiological functions, ranging from development and tissue homeostasis, to immune responses^{1, 2}. IL-11 signalling requires a multimeric structure comprising two specific α chains (IL-11R α), two gp130 chains (common to the IL-6 signaling receptor), and 2 cytokines. This hexameric complex



allows juxtaposition of the intracellular Janus kinases (JAK) and activation of the signal transducer and activator of transcription 3 (STAT3)^{1,3}. While gp130 is ubiquitously expressed, IL-11R α expression is cell-specific. Soluble IL-11R α resulting from IL-11R proteolysis allows trans-signaling and widens the number of target cells⁴. Importantly, IL-11, like IL-6, has no affinity toward gp130 in the absence of its specific receptor α chain¹.

1. Garbers C. & Scheller J., 2013. Interleukin-6 and interleukin-11: Same same but different. *Biol. Chem.* 394:1145.
2. Kang S. et al., 2020. Historical overview of the interleukin-6 family cytokine. *J. Exp. Med.* 217: e20190347.
3. Morris R. et al., 2018. The molecular details of cytokine signaling via the JAK/STAT pathway. *Protein Science.* 27:1984.
4. Lamertz L. et al., 2018. Soluble gp130 prevents interleukin-6 and interleukin-11 cluster signaling but not intracellular autocrine responses. *Science Sign.* 11(550):eaar7388.

PRODUCT DESCRIPTION

HEK-Blue™ IL-11 cells were engineered from the human embryonic kidney HEK 293 cell line to detect bioactive human and murine IL-11 by monitoring the activation of the JAK/STAT pathway. These cells can also be used for screening anti-IL-11R α antibodies using flow cytometry. HEK-Blue™ IL-11 cells were generated by stable overexpression of the human IL-11R α , human STAT3, and a STAT3-inducible secreted embryonic alkaline phosphatase (SEAP) reporter. Binding of IL-11 to its receptor on the surface of HEK-Blue™ IL-11 cells triggers a signaling cascade leading to the activation of STAT3 and production of SEAP. This can be readily assessed using QUANTI-Blue™ Solution. Of note, although HEK-Blue™ IL-11 cells are also knock-out for the IL-6R gene, they display a residual response to IL-6.

HEK-Blue™ IL-11 cells are resistant to Blastocidin, Hygromycin B and Zeocin™.

Detection range for human & murine IL-11: 100 pg/ml - 100 ng/ml

TECHNICAL SUPPORT

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Any questions about our cell lines?

Visit our FAQ page.

 **InvivoGen**
www.invivogen.com

SAFETY CONSIDERATIONS

Biosafety level 2

HEK-Blue™ IL-11 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 HEK-Blue™ Selection 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 Selection 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. **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% CO₂.

Frozen Stock Preparation

1. Resuspend cells at a density of 3-5 x 10⁶ 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™ IL-11 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 1X HEK-Blue™ Selection.
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™ IL-11 cells is ~24 hours using the conditions described above.

DETECTION OF IL-11

Day 1

1. Prepare HEK-Blue™ IL-11 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 10⁵ cells/ml.

Note: The response of HEK-Blue™ IL-11 cells can be altered by the action of trypsin. Do not use trypsin to detach HEK-Blue™ IL-11 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 IL-11 (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™ IL-11 cell suspension (~5 x 10⁴ cells) per well.
5. Incubate overnight at 37 °C in 5% CO₂.

Day 2

6. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed product data sheet.
7. Add 20 µl of induced HEK-Blue™ IL-11 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.

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 medium	rep-qbs

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