

HEK-Blue™ IL-10 Cells

Human & Murine IL-10 Reporter Cells

Catalog code: hkb-il10

<https://www.invivogen.com/hek-blue-il10>

For research use only

Version 19K14-NJ

PRODUCT INFORMATION

Contents

- 3-7 x 10⁶ HEK-Blue™ IL-10 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. Store at 4°C or at -20°C.*

*The expiry date is specified on the product label.

- 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 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 of 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 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

- SEAP reporter activity in response to human IL-10 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

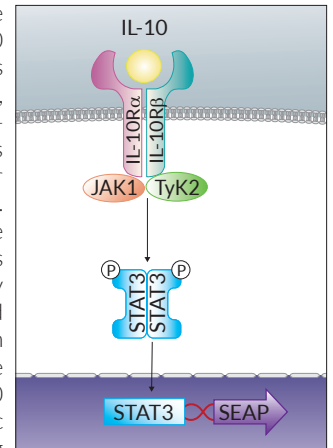
These cells are distributed for research purposes only.

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For non-research use, such as screening, quality control or clinical development, contact info@invivogen.com.

BACKGROUND

Interleukin 10 (IL-10), is the founding member of the IL-10 cytokine family, which comprises IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28A (IFN- λ 2), IL-28B (IFN- λ 3), and IL-29 (IFN- λ 1)¹. IL-10 was originally depicted as a key mediator of anti-inflammatory responses. While the immunosuppressive action of IL-10 is illustrated by its genetic association to inflammatory bowel disease, or its upregulated expression in chronic infections, an unanticipated IL-10 protective role has been reported in cancer¹. IL-10 also plays important homeostatic roles in non-immune cells, including neurons, adipocytes, and epithelial cells¹. The manipulation of IL-10 production and signaling has thus become an attractive therapeutic strategy². Upon IL-10 binding, the IL-10R α chain oligomerizes with the IL-10R β chain. This in turn allows the phosphorylation of JAK1 (Janus kinase 1) and Tyk2 (Tyrosine kinase 2), and the subsequent activation of STAT3 (Signal transducer and activator of transcription 3)¹. Importantly, STAT3 is also activated downstream of other cytokine receptors. How the cells trigger distinct and specific gene expression in response to cytokines signaling through the same transcription factor is not fully understood yet¹.



1. Saraiva M, et al., 2019. Biology and therapeutic potential of interleukin-10. J. Exp. Med. doi: 10.1084/jem.20190418. 2. Ouyang W & O'Garra A, 2019. IL-10 family cytokines IL-10 and IL-22: from basic science to clinical translation. Immunity, 50:871-91.

PRODUCT DESCRIPTION

HEK-Blue™ IL-10 cells were designed for the detection of bioactive human IL-10 (hIL-10) by monitoring the activation of the STAT3 pathway. HEK-Blue™ IL-10 cells were generated by stable transfection of the human embryonic kidney HEK293 cell line with the genes encoding hIL-10R α and β chains, human STAT3, and the SEAP (secreted embryonic alkaline phosphatase) reporter gene. The reporter gene is placed under the control of the IFN- β minimal promoter fused to AP1- and STAT3-binding sites. Because STAT3 is also implicated in the signaling of cytokines that do not belong to the IL-10 family, such as IFN- α / β and IL-6, HEK-Blue™ IL-10 cells are knockout for the expression of hIFNAR2 and hIL-6R. Binding of IL-10 to its receptor on the surface of HEK-Blue™ IL-10 cells triggers JAK1/STAT3 signaling. The subsequent production of SEAP in the culture supernatant can be readily assessed using QUANTI-Blue™ Solution. HEK-Blue™ IL-10 cells are resistant to Blastidicin, Hygromycin B and Zeocin™.

Detection range for human IL-10: 0.3-30 ng/ml

TECHNICAL SUPPORT

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www.invivogen.com

SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ IL-10 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% 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 both Normocin™ and HEK-Blue™ Selection**

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 to a larger tube containing 15 ml of pre-warmed growth medium. **Do not add selection antibiotics until the cells have been passaged twice.**
4. Centrifuge at 1000-1200 RPM (RCF 200-300 xg) 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 10⁶ cells/ml in freshly prepared freezing 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™ IL-10 cells with less than 20 passages.
- Handling of cells should be as short as possible to prevent any damage resulting from a prolonged stay at room temperature without 5% CO₂.

Cell Maintenance

1. HEK-Blue™ IL-10 cells are adherent. To split the cell culture, 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.
Note: The response of HEK-Blue™ IL-10 cells can be altered by the action of trypsin. Do not use trypsin to detach HEK-Blue™ IL-10 cells.
2. Maintain and subculture the cells in growth medium supplemented with 1x **HEK-Blue™ Selection**.
3. Renew the 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.

REPORTER ASSAY

Day 1:

1. Prepare HEK-Blue™ IL-10 cell suspension at ~280,000 cells/ml in fresh, pre-warmed test medium.
2. Add 20 µl of each sample per well of a flat-bottom 96-well plate.
3. Add 20 µl of recombinant human IL-10 at 10 ng/ml (positive control) in one well.
4. Add 20 µl of a recombinant cytokine such as **recombinant human TNF-α** 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™ IL-10 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

Product	Description	Cat. Code
HEK-Blue™ Selection	Selection antibiotics	hb-sel
Normocin™	Antimicrobial Reagent	ant-nr-1
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
Recombinant human TNF-α	Recombinant cytokine	rcyc-htnfa

TECHNICAL SUPPORT

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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, rep-qbs3

<https://www.invivogen.com/quant-blue>

For research use only

Version 20C16-MM

PRODUCT INFORMATION

Contents: QUANTI-Blue™ Solution is available in three pack sizes

- **rep-qbs:** 5 x 1 ml of QB reagent and 5 x 1 ml QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **25 x 96-well plates** (500 ml using the standard procedure) or **20 x 1536-well plates** (85 ml using the HTS screening procedure).

- **rep-qbs2:** 10 x 1 ml of QB reagent and 10 x 1 ml QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **50 x 96-well plates** (1 L using the standard procedure) or **40 x 1536-well plates** (170 ml using the HTS screening procedure).

- **rep-qbs3:** 1 x 20 ml bottle of QB reagent and 1 x 20 ml bottle of QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **100 x 96-well plates** (2 L using the standard procedure) or **80 x 1536-well plates** (340 ml using the HTS screening procedure).

Required Material (not provided)

- Sterile water
- Sterile screw cap tube, glass bottle or flask

Storage and stability

- Product is shipped at room temperature. Upon receipt, store QB reagent and QB buffer at -20°C. Product is stable for 1 year at -20°C when properly stored.

- The 20 ml bottles of QB reagent and QB buffer are designed for single use. If required, individual aliquots of QB reagent and QB buffer can be prepared upon receipt or following a single freeze-thaw cycle. Store aliquots at -20°C. **Avoid repeated freeze-thaw cycles.**

Note: During storage, a precipitate may form in the 20 ml bottle of QB reagent. If this occurs, vortex the product until the precipitate disappears. The formation of a precipitate does not affect the activity of the product.

- 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 glycosylphosphatidylinositol (GPI)-anchored protein. SEAP is secreted into the cell culture supernatant and therefore offers many advantages over intracellular reporters.

QUANTI-Blue™ is highly sensitive for quantitative measurement. It has a higher saturation threshold than with pNPP (p-nitrophenyl phosphate) resulting in more significant differences between no, low or high AP activity. Another advantage of QUANTI-Blue™ is that it can determine secreted AP activity without disturbing cells, thus allowing the repeated sampling of cell cultures for kinetic studies.

TECHNICAL SUPPORT

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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

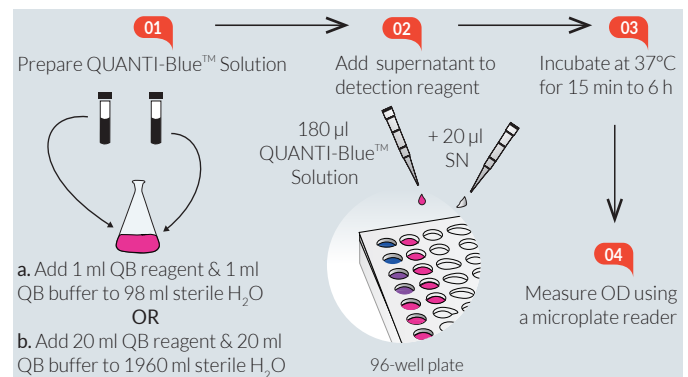


Figure 1. Standard procedure using QUANTI-Blue™ Solution.

The following protocol refers to the use of 96-well plates. Ensure QB reagent and QB buffer are completely thawed before use.

Note: For fast thawing, QB reagent and QB buffer can be placed at 37°C for 2 minutes. Ensure heating at 37°C does **not** exceed 5 minutes.

1. In a sterile bottle or flask, prepare QUANTI-Blue™ Solution by adding:
 - a. 1 ml of QB reagent and 1 ml of QB buffer to 98 ml of sterile water.
 - b. 20 ml of QB reagent and 20 ml of QB buffer to 1960 ml of sterile water.
2. Mix 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. Dispense 180 µl of QUANTI-Blue™ Solution per well into a flat-bottom 96-well plate.
5. Add 20 µl of the sample (supernatant of SEAP-expressing cells) or negative control (cell culture medium).
6. Incubate at 37°C for 15 min to 6 h.
7. Measure optical density (OD) at 620-655 nm using a microplate reader.

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

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

	96-well plate	24-well plate	12-well plate
QUANTI-Blue™	180 µl	450 µl	900 µl
Supernatant	20 µl	50 µl	100 µl

B. High Throughput Screening (HTS) procedure



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

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.
Note: For fast thawing, QB reagent and QB buffer can be placed at 37°C for 2 minutes. Ensure heating at 37°C does **not** exceed 5 minutes.

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 QUANTI-Blue™ Solution by adding:
 - a. **1 ml** of QB reagent and **1 ml** of QB buffer to **15 ml** of sterile water in a sterile 50 ml screw cap tube.
 - b. **20 ml** of QB reagent and **20 ml** of QB buffer to **300 ml** of sterile water in a sterile glass bottle or flask.
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 $\leq 5 \mu\text{l}$ 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 heating FBS at 56°C for 30 min to inactivate the alkaline phosphatase activity.

RELATED PRODUCTS

Product	Catalog Code
pNifTy2-SEAP (Zeo®)	pnifty2-seap
pSELECT-zeo-SEAP	psetz-seap
HEK-Blue™ Detection	hb-det2
Recombinant SEAP Protein	rec-hseap
Reporter cells	
HEK-Blue™ hTLR2	hkb-htlr2
HEK-Blue™ hTLR4	hkb-htlr4
RAW-Blue™ Cells	raw-sp
THP1-Blue™ NF-κB Cells	thp-nfkb
THP1-Blue™ ISG Cells	thp-isg

For a complete list of InvivoGen's Reporter Cell Lines visit <https://www.invivogen.com/reporter-cells>

TECHNICAL SUPPORT

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