HEK-Blue™ IL-17 Cells

Human IL-17A/IL-17F and human & mouse IL-17E reporter cells

Catalog code: hkb-il17

https://www.invivogen.com/hek-blue-il17

For research use only

Version 23K27-MM

PRODUCT INFORMATION

Contents

- 3-7 x 10^6 of HEK-Blue™ IL-17 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 the required selection antibiotics. HEK-Blue™ Selection can be stored at 4 °C or at -20 °C.*
- 1 ml 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'.

Quality Control

- SEAP reporter activity in response to various cytokines has been validated using functional assays.
- The stability for 20 passages following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

Cell Line Stability

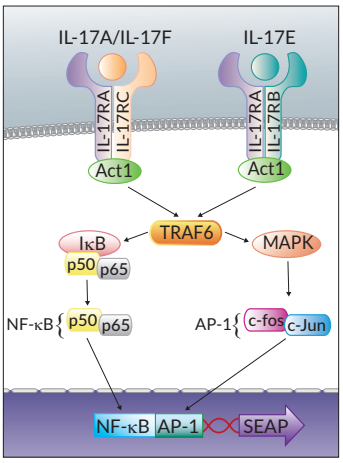
Cells will undergo genotypic changes resulting in reduced responsiveness over time in normal cell culture conditions. Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages. HEK-Blue™ IL-17 cells should be maintained in growth medium containing HEK-Blue™ Selection and Normocin™.

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.

BACKGROUND



Interleukin 17 (IL-17) is a family of six closely related cytokines (IL-17A to IL-17F) whose biological effects are mediated by binding to a heterodimeric receptor consisting of the ubiquitous IL-17RA and a second cytokine-specific receptor[1,2]. Of note, a number of family members, including IL-17A and IL-17F, play an important role in Th17 immunity and are implicated in tumorigenesis and autoimmune diseases[1,2]. Whereas IL-17E (also known as IL-25) appears to promote Th2 immunity[1]. Interestingly, IL-17A and IL-17F can form a heterodimer, which alters their potencies[1]. Both IL-17A and IL-17F exert their biological effects by binding to the IL-17RA/IL-17RC heterodimer, while IL-17E binds to the IL-17RA/IL-17RB heterodimer[1,2]. Upon binding, these heterodimeric receptors recruit the adaptor Act1, which leads to TRAF6 ubiquitylation. This triggers a signaling cascade that results in NF-κB and AP-1 activation[1].

1. Monin L. & Gaffen S.L., 2018. Interleukin 17 family cytokines: signaling mechanisms, biological activities, and therapeutic implications. Cold Spring Harb Perspect Biol. 10(4). **2. Pappu R. *et al.*, 2011.** The interleukin-17 cytokine family: critical players in host defence and inflammatory diseases. Immunology. 134:8-16.

PRODUCT DESCRIPTION

HEK-Blue™ IL-17 cells are designed to detect bioactive human IL-17A (hIL-17A), hIL-17E (IL-25) and hIL-17F, by monitoring the activation of the NF-κB and AP-1 pathways. These cells can be used for screening anti-IL-17 and anti-IL17 receptor antibodies. They were generated by stably introducing the human genes for the IL-17RA/IL-17RC heterodimer and the Act1 adaptor molecule into HEK293 cells. HEK-Blue™ IL-17 cells also respond to murine IL-17E (mIL-17E) and display little to no response to mIL-17A and mIL-17F. They do not respond to human nor murine IL-17C. Due to the TNF receptor and MyD88 gene knockout, these cells do not respond to TNF-α or IL-1β. These cells express a SEAP reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB and five AP-1 binding sites. Binding of IL-17A, IL-17E or IL-17F to the corresponding receptor on the surface of HEK-Blue™ IL-17 cells triggers a signaling cascade leading to the activation NF-κB and AP-1 and production of SEAP. This can be readily assessed using QUANTI-Blue™ Solution. HEK-Blue™ IL-17 cells are resistant to blasticidin, hygromycin B and Zeocin®.

Detection range for hIL-17A, hIL-17E & mIL-17E: 1 - 100 ng/ml
Detection range for hIL-17F: 3 - 100 ng/ml

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

InvivoGen
www.invivogen.com

SAFETY CONSIDERATIONS

HEK-Blue™ IL-17 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) fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml Normocin™
• Test Medium: DMEM, 4.5 g/l glucose, 2 mM L-Glutamine, 10% (v/v) heat-inactivated FBS (30 min at 56 °C), 100 U/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml Normocin™
• Freezing Medium: DMEM with 20% (v/v) FBS and 10% (v/v) DMSO

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 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 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% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 3-5 x 10^6 cells/ml in freshly prepared freezing medium with cold growth medium.

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 Handling Recommendations

To ensure the best results:

- Use HEK-Blue™ IL-17 cells with less than 20 passages.

Cell Maintenance

1. HEK-Blue™ IL-17 cells grow as adherent cells. Detach the cells using trypsin for 2-3 min at room temperature (RT).

Note: Prolonged action of trypsin or incubation at 37°C may alter the cell surface expression of receptors.

2. Maintain and subculture the cells in growth medium containing 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 cell grow to 100% confluency.

DETECTION OF IL-17

Sample preparation

- Warm the samples to 37 °C before use.

Note: Make sure that your samples do not contain alkaline phosphatase activity as it may interfere with the SEAP detection assay.

Day 1

1. Prepare HEK-Blue™ IL-17 cell suspension: gently rinse cells twice with pre-warmed phosphate buffered saline (PBS), detach the cells in the presence of PBS for 2-3 min at 37°C. Tap the flask if needed. Resuspend cells in fresh, pre-warmed test medium and prepare a cell suspension at ~280,000 cells/ml.

Note: We recommend avoiding the use of trypsin to detach cells for the functional assays (see FAQs online).

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-17A (final concentration 10 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-17 cell suspension (~50,000 cells) per well.
5. Incubate overnight at 37 °C in 5% CO_2.

Day 2

6. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed product data sheet.
7. Add 180 µl of resuspended QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
8. Add 20 µl of induced HEK-Blue™ IL-17 cells supernatant.
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™ IL-17C Cells	IL-17C reporter cells	hkb-il17c
HEK-Blue™ Selection	Antibiotic mixture	hb-sel
Normocin™	Antimicrobial reagent	ant-nr-1
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs
Recombinant human TNF-α	Recombinant hTNF-α	rcyc-htnfa

TECHNICAL SUPPORT
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InvivoGen Asia: +852 3-622-34-80
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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/quanti-blue

For research use only

Version 23C09-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 and QB buffer. If this occurs, heat the product at 37°C for 30 seconds and vortex 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 from light.

Quality Control

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.

- Physicochemical characterization (pH, appearance).
- 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.

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

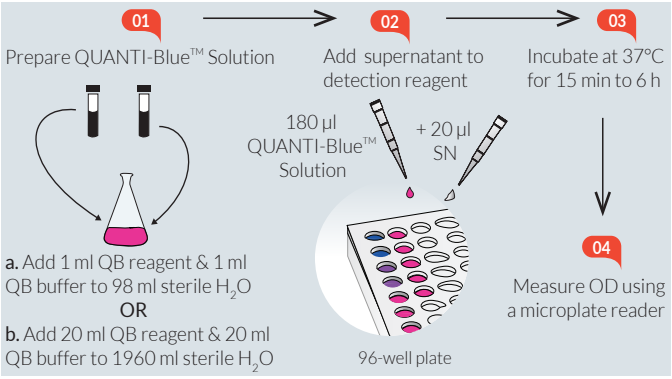

Figure 1. Standard procedure using QUANTI-Blue™ Solution.

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

TECHNICAL SUPPORT
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E-mail: info@invivogen.com

B. High Throughput Screening (HTS) procedure

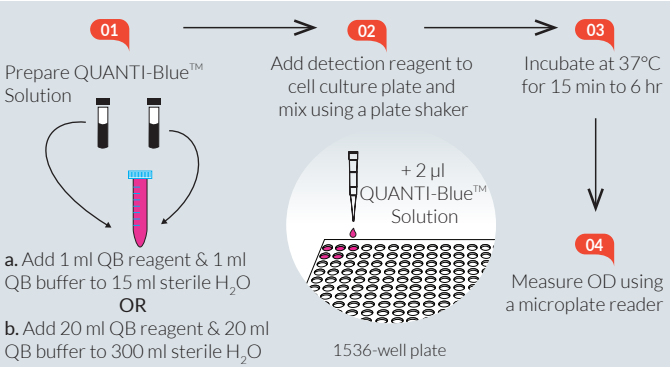

Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

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 ≤ 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 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