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 19G30-MM

PRODUCT INFORMATION

Contents

- **1 vial of HEK-Blue™ IL-17 cells** (3-7 x 10^6 cells)
- **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 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 Cells Upon Receipt

Cells must be thawed **immediately** upon receipt and grown according to handling procedures (as described on the next page), to ensure cell viability and proper assay performance.

*Note: **Do not freeze the 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.

Quality Control

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

Cell Line Stability

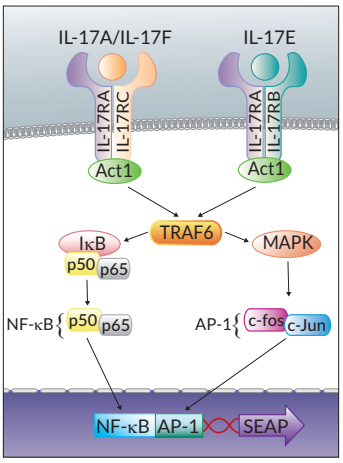
Cells will undergo genotypic changes resulting in reduced responsiveness over time in normal cell culture conditions. Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages. 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-kB 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-kB 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
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@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 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 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. Maintain and subculture the cells in growth medium containing 1x HEK-Blue™ Selection.
2. Renew growth medium twice a week.
3. Cells should be passaged when a 70-80% confluency is reached, detach the cells in the presence of phosphate buffered saline (PBS) by tapping the flask or by using a cell scraper. Do not let the cells grow to 100% confluency.

Note: The response of HEK-Blue™ IL-17 cells can be altered by the action of trypsin. Do not use trypsin to detach HEK-Blue™ IL-17 cells.

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 PBS, detach the cells in presence of PBS by tapping the flask or by using a cell scraper, resuspend cells in fresh, pre-warmed test medium (containing heat-inactivated FBS) and prepare a cell suspension at 3 x 10^5 cells/ml.

Note: Some FBS may contain alkaline phosphatases that can interfere with SEAP quantification. We recommend to use heat-inactivated FBS to inactivate these enzymes which are thermosensitive.

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™ 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
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

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

For research use only

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 therefore offers many advantages over intracellular reporters.

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

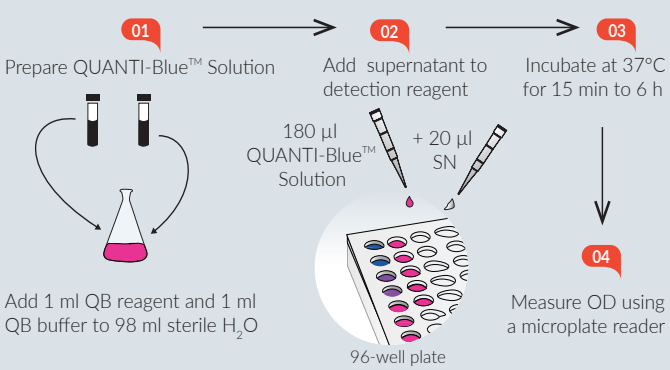


Figure 1. Standard procedure using QUANTI-Blue™ Solution.

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. Prepare 100 ml of QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml of 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. Dispense 180 µl of QUANTI-Blue™ Solution per well into a flat-bottom 96-well plate.
5. Add 20 µl of 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 to heat 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
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com

B. High Throughput Screening (HTS) procedure

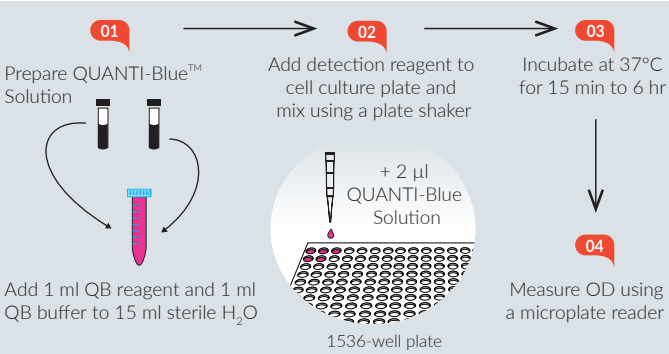


Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

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 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.

RELATED PRODUCTS

Product	Catalog Code
pNiFty2-SEAP (Zeo^R)	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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InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com