**PRODUCT INFORMATION**

**Contents**
- 1 vial of HEK-Blue™ IL-17 cells (3-7 x 10^6 cells) in freezing medium

**IMPORTANT:** Cells are shipped frozen. If cells are not frozen upon arrival, contact InvivoGen immediately.

- 2 x 1 ml 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), Normocin™ is a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20 ºC.*
- The expiry date is specified on the product label.
- 1 pouch of QUANTI-Blue™, a secreted embryonic alkaline phosphatase (SEAP) detection reagent. Store QUANTI-Blue™ pouch at 4 °C for 12 months. Reconstituted product is stable 2 weeks at 4°C. Protect QUANTI-Blue™ from light.

**Handling Cells Upon Receipt**

Cells must be thawed immediately upon receipt and grown according to handling procedures (as described overhead), 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**

- HEK-Blue™ IL-17 cells were stimulated with various cytokines (IL-1β, IL-17A, IL-17E, IL-17F and TNF-α). Production of SEAP was detected only following stimulation by IL-17A, IL-17E, IL-17F.
- 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™. Antibiotic pressure with HEK-Blue™ Selection is required to maintain the plasmids coding for the adaptor molecule Act1, the IL-17 receptor and the SEAP reporter.

**BACKGROUND**

Interleukin 17 (IL-17) is a family of six cytokines; including the pro-inflammatory IL-17A, IL-17E (also known as IL-25) and IL-17F, and the poorly understood IL-17B, IL-17C, and IL-17D. IL-17A, IL-17E and IL-17F bind a heterodimeric surface receptor (IL-17RA and IL-17RC), which recruits the adaptor Act1 leading to TRAF6 ubiquitylation, triggering a signaling cascade that results in AP-1 and NF-κB activation. These cytokines exert a protective role against many pathogens, but are associated with autoimmune diseases when overexpressed.


**PRODUCT DESCRIPTION**

HEK-Blue™ IL-17 cells are designed to detect bioactive human (hIL-17) and murine IL-17 (mIL-17) by monitoring the activation of the NF-κB and AP-1 pathways. These cells were generated by stably introducing the human genes for the IL-17RA/IL-17RC heterodimer and the Act1 adaptor molecule into HEK293 cells. Furthermore, in these cells the TNF-α and MyD88-dependent IL-1β responses are blocked, hence, HEK-Blue™ IL-17 cells respond specifically to IL-17.

HEK-Blue™ IL-17 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-17 to IL-17RA/IL-17RC on the surface of HEK-Blue™ IL-17 cells triggers a signaling cascade leading to the activation NF-κB and AP-1 with the subsequent production of SEAP. Detection of SEAP in the supernatant of HEK-Blue™ IL-17 cells can be readily assayed using QUANTI-Blue™, a SEAP detection reagent. QUANTI-Blue™ turns blue in the presence of SEAP, which can be easily quantified using a spectrophotometer.

**Detection range for hIL-17A & mIL-17A:** 300 pg - 100 ng/ml
**Detection range for hIL-17F:** 300 pg - 100 ng/ml
**Detection range for hIL-17E:** 1 ng - 100 ng/ml
SAFETY CONSIDERATIONS
HEK-Blue™ IL-17 cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) that require Biosafety level 2 according to CDC guidelines. The biosafety level may vary depending on the country.

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), 50 U/ml penicillin, 50 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), 50 U/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml Normocin™
Note: Heat-inactivated FBS is also commercially available.
- Freezing Medium: DMEM with 20% (v/v) fetal bovine serum 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 cell 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 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 (Nalgene) 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 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 ~280,000 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 (0.25 µg/ml), and 20 µl of a negative control, such as recombinant human TNF-α (0.25 µg/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% CO2.

Day 2
6- Prepare QUANTI-Blue™ following the instructions on the enclosed product data sheet.
7- Add 180 µl of resuspended QUANTI-Blue™ 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.

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 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

RELATED PRODUCTS

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<th>Product</th>
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<td>Normocin™</td>
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<tr>
<td>QUANTI-Blue™</td>
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TECHNICAL SUPPORT
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InvivoGen Europe: +33 (0) 5-62-71-69-39
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QUANTI-Blue™
Medium for detection and quantification of alkaline phosphatase
Catalog # rep-qb1, rep-qb2
For research use only
Version # 16C18-MM

PRODUCT INFORMATION
Contents:
QUANTI-Blue™ is provided as packs of individually sealed pouches.
• rep-qb1: 5 pouches of QUANTI-Blue™
• rep-qb2: 10 pouches of QUANTI-Blue™
Each pouch contains everything needed to prepare 100 ml of medium for the detection and quantification of any alkaline phosphatase.

Storage and Stability:
- Store QUANTI-Blue™ pouches at 2-8 °C for 12 months.
Important: The correct storage temperature for this product is 2-8 °C (some pouches may be mislabeled).
- Reconstituted QUANTI-Blue™ medium is stable 2 weeks at 2-8 °C and 2 months at -20°C. Keep reconstituted QUANTI-Blue™ away from light.

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™ medium changes 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 that are exploited by the use of QUANTI-Blue™.

• Requires small samples of cell supernatants - Samples of 10 µl are sufficient.
• No need to process samples - Preparation of cell lysates or heating of samples are not required.
• Determine secreted AP activity without disturbing cells - The same cell cultures can be repeatedly sampled for kinetic studies or further experimentation.
• 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™.
• 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 resulting in more significant differences between non or low AP expression and high AP expression.
• Extremely simple to use - QUANTI-Blue™ consists of only one medium: 1) resuspend in water, 2) add sample, incubate at 37°C and 3) assess AP activity with the naked eye or by reading the optical density (OD) at 625-655 nm.

METHODS
Preparation of QUANTI-Blue™
- Pour the contents of one pouch of QUANTI-Blue™ in a 250 ml sterile glass bottle or flask.
- Add 100 ml of endotoxin-free water.
- Swirl gently.
- Warm QUANTI-Blue™ to 37°C for 30 min.
- Use reconstituted QUANTI-Blue™ immediately or store at 2-8 °C.

Notes:
- QUANTI-Blue™ may require overnight incubation at 2-8°C to ensure complete dissolution of the powder.
- Optional: To guarantee sterility, QUANTI-Blue™ can be filtered on a 0.2 µm membrane once complete dissolution is achieved. However, this step is not necessary as your cells will not be in contact with QUANTI-Blue™.

Detection of SEAP activity from cell culture supernatants
The following protocol refers to the use of 96-well plates. Vary your procedure accordingly depending on volumes of reagents needed based on the size of your wells. Some fetal bovine serum (FBS) may contain alkaline phosphatase that can interfere with SEAP quantification. We recommend to test the culture medium supplemented with FBS as a negative control to evaluate the presence of alkaline phosphatase in the serum.

- Aliquot 200 µl QUANTI-Blue™ per well.
Note: Warm QUANTI-Blue™ to 37 °C before use.
- Add 20 µl supernatant of SEAP-expressing cells or cell culture medium as a negative control.
Note: If the negative control turns purple/blue, it means your FBS contains alkaline phosphatase. We recommend to heat the FBS used in your cell culture medium at 56 °C for 30 minutes to inactivate the alkaline phosphatase activity.
- Incubate at 37 °C.
- After 15 min to 24 h incubation, assess SEAP activity with the naked eye or by reading the OD at 620-655 nm with a microplate reader.

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<td>Recombinant SEAP Protein</td>
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