HEK-Blue™ IL-1R Cells
Human & Murine IL-1 Reporter Cells
Catalog code: hkb-il1r
https://www.invivogen.com/hek-blue-il1r
For research use only
Version 19H27-MM

PRODUCT INFORMATION
Contents
- 1 vial of HEK-Blue™ IL-1R cells (3-7 x 10⁶ cells)
- 1 ml of Hygromycin B Gold (>90% pure hygromycin B) at 100 mg/ml. Store at 4°C or -20°C.*
- 1 ml of Puromycin (10 mg/ml). Store at 4°C or -20°C.*
- 1 ml of Zeocin™ (100 mg/ml). Store 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.

Handling 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.

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 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.

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 with 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.

APPLICATIONS
These cells can be used to detect human and murine IL-1 cytokines (both IL-1α and IL-1β).
Note: HEK-Blue™ IL-1R do not respond to human TNF-α but do respond to murine TNF-α.

PRODUCT DESCRIPTION
HEK-Blue™ IL-1R cells were designed to detect bioactive human and murine IL-1β by monitoring the activation of the NF-κB and AP-1 pathways. Additionally, these cells detect bioactive IL-1β from cynomolgous monkeys, dogs, and rats. In fact HEK-Blue™ IL-1R cells can detect IL-1α and IL-1β, as these cytokines bind to the same receptor, IL-1R1. These cells derive from HEK-Blue™ IL-1β cells in which the TNF-α response is blocked. Therefore, HEK-Blue™ IL-1R cells respond specifically to IL-1. HEK-Blue™ IL-1R cells endogenously express the human IL-1 receptor and were stably transfected with the murine IL-1 receptor rendering these cells very sensitive to both human and murine IL-1β.

HEK-Blue™ IL-1R 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-1β to IL-1R1 on the surface of HEK-Blue™ IL-1R cells triggers a signaling cascade leading to the activation NF-κB and the subsequent production of SEAP. Detection of SEAP in the supernatant of HEK-Blue™ IL-1R cells can be readily assessed using QUANTI-Blue™ Solution, a SEAP detection reagent. QUANTI-Blue™ Solution turns blue in the presence of SEAP, which can be easily quantified using a spectrophotometer.

Detection range for hIL-1α/β: 1 pg - 100 ng/ml
Detection range for mIL-1α/β: 1 pg - 100 ng/ml

Figure 1: IL-1β-induced NF-κB signaling pathway.
SAFETY CONSIDERATIONS

HEK-Blue™ IL-1R 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), 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, 100 U/ml penicillin, 100 µg/ml streptomycin without Hygromycin B Gold, Normocin™, puromycin, and Zeocin™
• Freezing Medium: DMEM with 20% (v/v) FBS and 10% (v/v) DMSO

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 discard.
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 freezing medium prepared extemporaneously 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.

Required Selection Antibiotics

• Hygromycin B Gold, puromycin and Zeocin™

Cell Handling Recommendations

To ensure the best results:
- Use HEK-Blue™ IL-1R cells with less than 20 passages.

Cell Maintenance

1. Maintain and subculture the cells in growth medium containing hygromycin B Gold (200 µg/ml), puromycin (1 µg/ml) and Zeocin™ (100 µg/ml).
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 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-1R cells can be altered by the action of trypsin. Do not use trypsin to detach HEK-Blue™ IL-1R cells.

DETECTION OF IL-1

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-1R cell suspension: wash 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 growth medium and prepare a cell suspension at ~3 x 10⁶ 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 thermostable.
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-1β (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-1R cell suspension (~50,000 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 180 µl of resuspended QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
8. Add 20 µl of sample 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

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<td>QUANTI-Blue™ Solution</td>
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<td>Recombinant human IL-1β</td>
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<tr>
<td>Recombinant human TNF-α</td>
<td>ant-nr-1</td>
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TECHNICAL SUPPORT
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InvivoGen USA (International): +1 (858) 457-5873
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InvivoGen www.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

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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. Secretned 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 reporter gene. It is a truncated form of placental alkaline phosphatase, a GPI-anchored protein. SEAP is secreted into cell culture supernatant reporter gene. It is a truncated form of placental alkaline phosphatase.

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

1. Prepare 100 ml of QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml 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.

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

<table>
<thead>
<tr>
<th></th>
<th>96-well plate</th>
<th>24-well plate</th>
<th>12-well plate</th>
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<tbody>
<tr>
<td>QUANTI-Blue™</td>
<td>180 µl</td>
<td>450 µl</td>
<td>900 µl</td>
</tr>
<tr>
<td>Supernatant</td>
<td>20 µl</td>
<td>50 µl</td>
<td>100 µl</td>
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B. High Throughput Screening (HTS) procedure

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.

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For a complete list of InvivoGen’s Reporter Cell Lines visit [https://www.invivogen.com/reporter-cells](https://www.invivogen.com/reporter-cells)