HEK-Blue™ IL-22 Cells

Human & Murine IL-22 Reporter Cells

Catalog code: hkb-il22

https://www.invivogen.com/hek-blue-il22

For research use only

Version 23B27-MM

PRODUCT INFORMATION

Contents

- **3-7 x 10^6 of HEK-Blue™ IL-22 cells** (3-7 x 10^6 cells)
- **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 Blasticidin (10 mg/ml).** Store at 4 °C or at -20°C.*
- **1 ml of Puromycin (10 mg/ml).** Store at 4°C or at -20 °C.*
- **1 ml of Zeocin™ (100 mg/ml).** Store at 4°C or 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 IL-22 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.

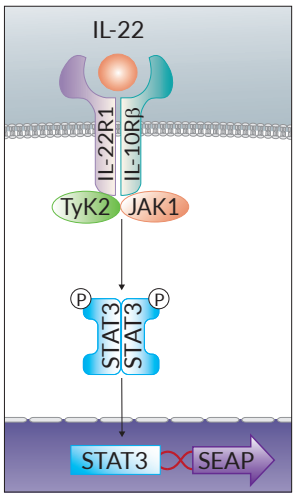
USE RESTRICTIONS

These cells are distributed for research purposes only.

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.

BACKGROUND



Interleukin 22 (IL-22) is a key regulator of immunity and inflammation at mucosal surfaces. Its principal role is to maintain barrier integrity against pathogens[1-3]. IL-22 production can be triggered by a variety of pathogen-associated molecular patterns (PAMPs). Notably, it can be induced directly by TLR2 receptor activation in response to bacterial pathogens or indirectly via IL-23 in response to aryl-hydrocarbon receptor (AhR) ligands[1,2]. IL-22 is implicated in a number of pathologies including autoimmune diseases and cancer[3,4]. IL-22 exerts its biological effect upon binding to its receptor, which comprises two subunits: IL-22R1 and IL-10Rβ. Upon binding, IL-22 triggers a signaling pathway involving tyrosine kinase 2 (TyK2) and Janus kinase 1 (JAK1) leading to the activation of signal transducer and activator of transcription 3 (STAT3)[1].

1. Wang J. *et al.*, 2018. Aryl hydrocarbon receptor/IL-22/Stat3 signaling pathway is involved in the modulation of intestinal mucosa antimicrobial molecules by commensal microbiota in mice. Innate Immun. 24(5):297-306. **2. Foxall R.B. *et al.*, 2016.** Profile of interleukin-22 in gut mucosal health and disease. IJICMR. 8:1-11. **3. Zenewicz L.A., 2018.** IL-22: There Is a Gap in Our Knowledge. Immunohorizons. 2(6):198-207. **4. Hernandez P. *et al.*, 2018.** A catch-22: Interleukin-22 and cancer. Eur J Immunol. 48(1):15-31.

PRODUCT DESCRIPTION

HEK-Blue™ IL-22 cells are designed for the detection of bioactive human (hIL-22) and murine IL-22 (mIL-22) by monitoring the activation of the STAT3 pathway. These cells can be used for screening anti-IL-22 antibodies. They were generated by stable transfection of the human embryonic kidney HEK293 cell line with the genes encoding the human IL-22 receptor, 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 four STAT3-binding sites. Of note, as HEK293 cells endogenously express the interferon-α/β receptor (IFNAR), these cells respond to type I IFNs.

Binding of IL-22 to its receptor on the surface of HEK-Blue™ IL-22 cells triggers a signaling cascade leading to the activation of STAT3 and the subsequent production of SEAP. This can be readily assessed using QUANTI-Blue™ Solution, a SEAP detection reagent.

HEK-Blue™ IL-22 cells are resistant to blasticidin, hygromycin B and Zeocin™.

Detection range for hIL-22: 0.03-10 ng/ml
Detection range for mIL-22: 0.1-10 ng/ml

TECHNICAL SUPPORT
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SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ IL-22 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 Normocin™, Blasticidin, Puromycin and Zeocin™**

Required Selection Antibiotics

- Blasticidin, Puromycin and Zeocin™

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 Blasticidin, Puromycin and Zeocin™ until the cells have been passaged twice.**
4. Centrifuge 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 cells to a T-25 culture flask containing 5 ml of growth medium.
7. Place the culture at 37 °C in 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10^6 cells/ml in freezing medium freshly prepared with cold DMEM.

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 Maintenance

1. HEK-Blue™ IL-22 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 supplemented with 10 µg/ml of blasticidin, 1 µg/ml of puromycin, and 100 µg/ml of Zeocin™.
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.

Cell Handling Recommendations

To ensure the best results:
- Use HEK-Blue™ IL-22 cells with less than 20 passages.

REPORTER ASSAY

Day 1:

1. Detach HEK-Blue™ IL-22 cells using trypsin for 2-3 min at room temperature (RT). Resuspend cells in fresh, pre-warmed test medium and prepare a cell suspension at ~280,000 cells/ml.

Note: The response of HEK-Blue™ IL-22 cells can be altered by the prolonged action of trypsin. Do not incubate with trypsin at 37°C and for no longer than 2-3 min.

2. Add 20 µl of each sample per well of a flat-bottom 96-well plate.
3. Add 20 µl of recombinant human or murine IL-22 at 1 ng/ml (positive control) in one well.
4. Add 20 µl of a recombinant cytokine such as recombinant human IL-6 at 1 ng/ml (negative control) in one well.

Note: For the negative control, do not use STAT3-activating cytokines.

5. Add 180 µl of cell suspension (~50,000 cells) per well.
6. Incubate the plate at 37 °C in a CO_2 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-22 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
Blasticidin	Selective antibiotic	ant-bl-1
Normocin™	Antimicrobial reagent	ant-nr-1
Puromycin	Selective antibiotic	ant-pr-1
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
Zeocin™	Selective antibiotic	ant-zn-1

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 23A12-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 (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

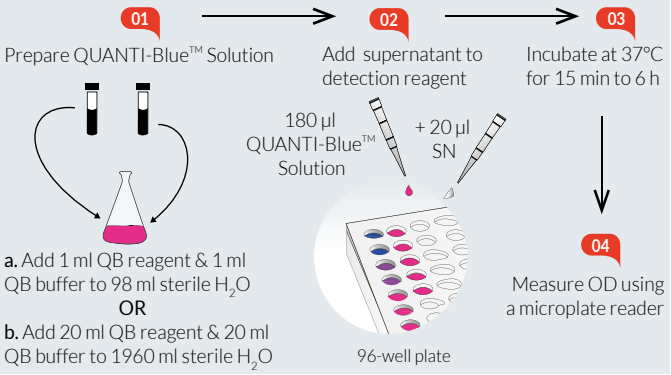

Figure 1. Standard procedure using QUANTI-Blue™ Solution.

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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B. High Throughput Screening (HTS) procedure

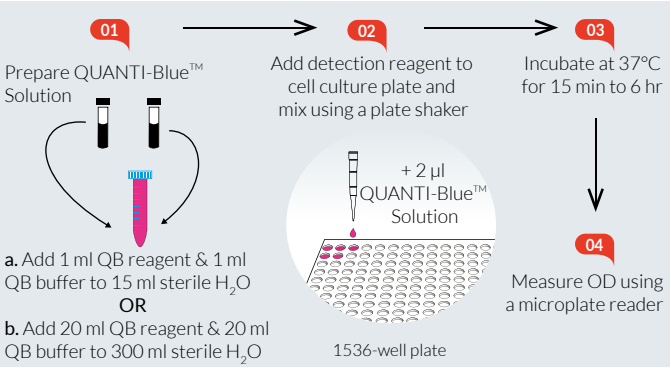

Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

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