

HEK-Blue™ IL-36 Cells

Interleukin-36 reporter cells

Catalog code: hkb-hil36r

<https://www.invivogen.com/hek-blue-il36>

For research use only

Version 23C07-MM

PRODUCT INFORMATION

Contents

• 3-7 x 10⁶ of HEK-Blue™ IL-36 cells in a cryovial or shipping flask.
IMPORTANT: If cells provided in a cryovial are not frozen upon arrival, contact InvivoGen immediately.

- 1 ml of Blasticidin (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.*
- 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.

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'.

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-36 cells should not be passaged more than 20 times to remain fully efficient.

Quality Control

- SEAP reporter activity in response to human IL-36 isoforms α , β and γ has been validated using functional assays.
- The expression of human IL-36R (IL-1R6) has been confirmed by RT-qPCR.
- The stability for 20 passages following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

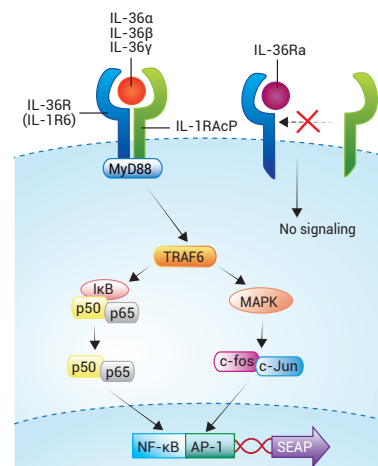
USE RESTRICTIONS

These cells are distributed for research purposes only.

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BACKGROUND

The cytokine interleukin 36 (IL-36) belongs to the IL-1 superfamily. Three isoforms, IL-36 α , IL-36 β and IL-36 γ , mediate pro-inflammatory functions, while a fourth one, IL-36Ra, acts as an antagonist^{1,2}. IL-36 signaling requires the formation of a complex comprised of the IL-36 receptor (IL-36R or IL-1R6) and the IL-1 receptor accessory protein (IL-1RAcP). The binding of agonist ligands to the IL-36R allows the recruitment of IL-1RAcP and the production of pro-inflammatory cytokines and chemokines through the activation of NF- κ B and AP-1^{1,2}. The IL-36Ra antagonist inhibits the signaling by binding to IL-36R and preventing the recruitment of IL-1RAcP^{1,2}. IL-36 associated immune response mainly takes place in barrier tissues, such as the skin, lungs, and intestines. Dysregulation of IL-36 isoform expression and signaling has been associated with inflammatory diseases such as psoriasis, rheumatoid arthritis, and inflammatory bowel disease^{1,2}.



1. Buhl A-L. & Wenzel J., 2019. Interleukin-36 in infectious and inflammatory skin diseases. *Front Immunol.* 10:1162.
2. Zhou L. & Todorovic V., 2021. Interleukin-36: Structure, Signaling and Function. *Adv Exp Med Biol.* 21:191-210.

PRODUCT DESCRIPTION

HEK-Blue™ IL-36 cells were engineered from the human embryonic kidney HEK 293 cell line to detect bioactive IL-36 by monitoring the activation of NF- κ B/AP-1. These cells can also be used for screening anti-IL-36R antibodies using flow cytometry. HEK-Blue™ IL-36 cells were generated by stable overexpression of the human IL-36R (IL-1R6), human IL-1RAcP, and a NF- κ B/AP-1-inducible secreted embryonic alkaline phosphatase (SEAP) reporter. Binding of IL-36 agonists to the IL-36R/IL-1RAcP dimeric receptor on the surface of HEK-Blue™ IL-36 cells triggers a signaling cascade leading to the activation of NF- κ B/AP-1 and production of SEAP. This can be readily assessed using QUANTI-Blue™ Solution. HEK-Blue™ IL-36 cells are resistant to Blasticidin and Zeocin®.

Detection range for human IL-36 α : 3 pg/ml - 10 ng/ml

Detection range for human IL-36 β : 1 ng/ml - 10 ng/ml

Detection range for human IL-36 γ : 3 pg/ml - 10 ng/ml

No detection for murine IL-36 α

TECHNICAL SUPPORT

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SAFETY CONSIDERATIONS

Biosafety level 2

HEK-Blue™ IL-36 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), Pen-Strep (100 U/ml - 100 µg/ml), 100 µg/ml **Normocin™**
- **Freezing Medium:** DMEM, 20% (v/v) FBS, 10% (v/v) DMSO
- **Test Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% (v/v) heat-inactivated FBS, Pen-Strep (100 U/ml - 100 µg/ml), **without Blastcidin, Zeocin® and Normocin™**

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

Required Selection Antibiotic(s)

- **Blasticidin** 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 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 mins.

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% CO₂.

Frozen Stock Preparation

1. Resuspend cells at a density of 3-5 x 10⁶ cells/ml in freshly prepared freezing medium with cold DMEM.

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.

Cell Maintenance

1. HEK-Blue™ IL-36 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 **Blasticidin** and 100 µg/ml of **Zeocin®**.

3. Renew 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.

Note: The average doubling time for the HEK-Blue™ IL-36 cells is ~24 hours using the conditions described above.

DETECTION OF IL-36

Day 1

1. Detach HEK-Blue™ IL-36 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-36 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 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-36α (final concentration 0.01 ng/ml), and 20 µl of a negative control, such as recombinant human IFN-α (final concentration 1000 U/ml).

4. Add 180 µl of HEK-Blue™ IL-36 cell suspension (~5 x 10⁴ 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 20 µl of induced HEK-Blue™ IL-36 cells supernatant.

8. Add 180 µl of resuspended **QUANTI-Blue™ Solution** 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

Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
Zeocin®	Selection antibiotic	ant-zn-1
Normocin™	Antimicrobial reagent	ant-nr-1
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs

TECHNICAL SUPPORT

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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/quant-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

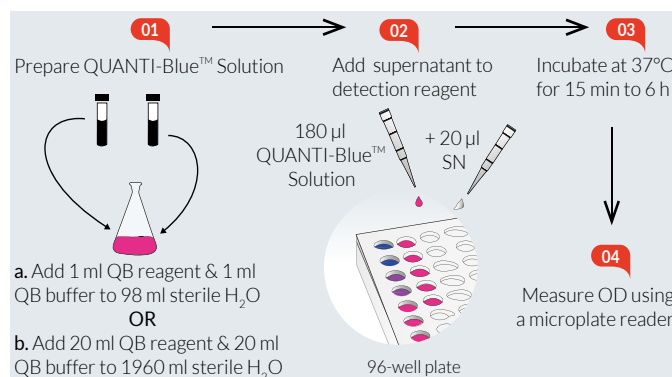


Figure 1. Standard procedure using QUANTI-Blue™ Solution.

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

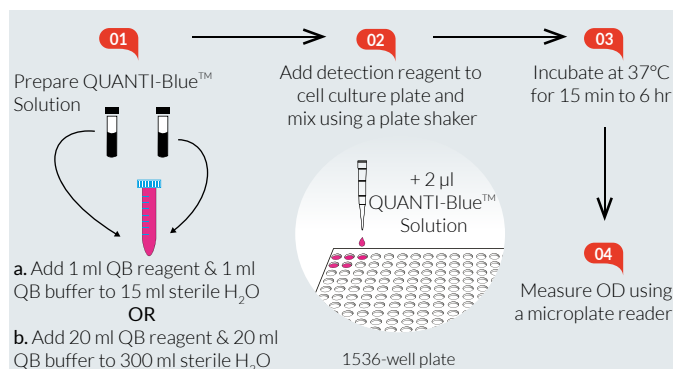


Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

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>

TECHNICAL SUPPORT

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