HEK-Blue™ IL-36 Cells

Interleukin-36 reporter cells

Catalog code: hkb-hil36r

invivogen.com/hek-blue-il36

For research use only

Version 25D18-NJ

PRODUCT INFORMATION

Contents

- **3-7 x 10^6 of HEK-Blue™ IL-36 cells** in a cryovial or shipping flask.

Note: 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 are shipped in dry ice, and upon receipt should immediately be thawed for culture or stored below -130°C, preferably in liquid nitrogen vapor, for long-term storage.

IMPORTANT: Do not store cell vials at -80°C as this will decrease cell viability and performance. Contact technical support if the cells are not frozen or in dry ice upon arrival.

To insure the highest level of viability and best assay performance, we strongly recommend that you thaw the cells and initiate the culture as soon as possible upon receipt (as described on the next page).

Warranties

- InvivoGen's cells are provided 'AS IS' and their viability is guaranteed upon shipment from our facilities for a period of 30 days, provided that the customer has properly stored and handled the product.
- Our cell lines are guaranteed free of mycoplasma contamination.
- The stability of our cell lines is guaranteed for 20 passages.

Quality Control

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

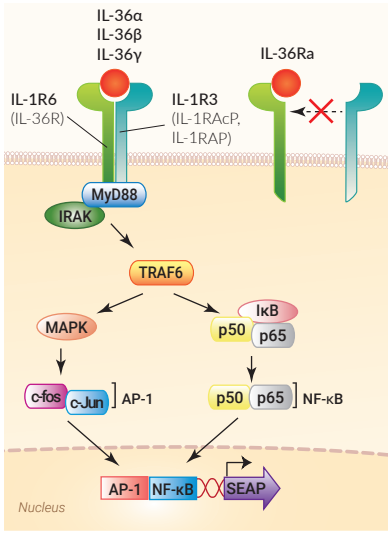
USE RESTRICTIONS

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 outlicensing@invivogen.com.

PRODUCT DESCRIPTION

HEK-Blue™ IL-36 cells were engineered from the human embryonic kidney HEK293 cell line to detect bioactive IL-36 by monitoring the activation of NF-κB/AP-1. These cells were generated by stable transfection with the genes encoding the human interleukin 36 receptor subunits, IL-1R6 and IL-1R3. In addition, an NF-κB/AP-1-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene was introduced. Stimulation of HEK-Blue™ IL-36 cells with IL-36 triggers a signaling cascade leading to the activation of NF-κB/AP-1 and the subsequent 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-36α: 30 pg/ml - 100 ng/ml
Detection range for human IL-36β: 10 ng/ml - 100 ng/ml
Detection range for human IL-36γ: 30 pg/ml - 100 ng/ml
No detection for murine IL-36α

BACKGROUND

The cytokine interleukin 36 (IL-36) belongs to the IL-1 superfamily. Three isoforms, IL-36α, IL-36β and IL-36γ, mediate pro-inflammatory functions, while a fourth one, IL-36Ra, acts as an antagonist[1,2]. IL-36 signalization requires the formation of a complex comprised of two subunits, IL-1R6 (aka IL-36R) and IL-1R3 (aka IL-1RAcP, IL-1 receptor accessory protein). The binding of agonist ligands to IL-1R6 allows the recruitment of IL-1R3 and the production of pro-inflammatory cytokines and chemokines through the activation of NF-κB and AP-1[1,2]. The IL-36Ra antagonist inhibits the signaling by binding to IL-1R6 and preventing the recruitment of IL-1R3[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.

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FAQ Any questions about our cell lines? Visit our FAQ page.

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www.invivogen.com

SAFETY CONSIDERATIONS

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 (HI) 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) HI FBS, Pen-Strep (100 U/ml-100 µg/ml) **without Normocin®, Blasticidin, and Zeocin®**

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. ***Warning: Do not add selection antibiotics until the cells have been passaged twice.***
4. Centrifuge vial 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 vial contents to a 25 cm^2 tissue culture flask containing 5 ml of growth medium without selection antibiotics.

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 5-7 x 10^6 cells/ml in freezing medium freshly prepared with cold growth medium.

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 Handling Recommendations

To ensure the best results, use HEK-Blue™ IL-36 cells with less than 20 passages.

Cell Maintenance

1. HEK-Blue™ IL-36 cells grow as adherent cells. Detach the cells using PBS at 37°C or trypsin at room temperature (RT) for 2-3 min.

Warning: Prolonged action of trypsin or incubation at 37°C may alter the cell surface expression of cytokine receptors.

2. Maintain and subculture the cells in growth medium supplemented with 10 µg/ml of 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.

DETECTION OF IL-36 ACTIVITY

We recommend to use **test medium** one passage prior to the assay.

Day 1:

1. Prepare HEK-Blue™ IL-36 cell suspension: gently rinse cells twice with pre-warmed phosphate buffered saline (PBS), detach the cells using PBS at 37°C or trypsin at room temperature (RT) for 2-3 min. Tap the flask if needed. Resuspend cells in fresh, pre-warmed test medium and prepare a cell suspension at ~280,000 cells/ml.
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 (0.1 ng/ml final concentration), and 20 µl of a negative control, such as recombinant human IFN-α (1000 U/ml final concentration).
4. Add 180 µl of HEK-Blue™ IL-36 cell suspension (~50,000 cells) per well.
5. Incubate overnight at 37 °C in 5% CO_2.

Day 2

1. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed product data sheet.
2. Add 20 µl of induced HEK-Blue™ IL-36 cells 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 30 min to 3 hours.
5. 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
Recombinant human IL-36α	Recombinant cytokine	rcyec-hil36a
Recombinant human IFN-α2b	Recombinant cytokine	rcyc-hifna2b
Anti-hIL-36R-hIgG4 (S228P)	Monoclonal antibody	hil36r-mab14

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