HEK-Blue™ IFN-α/β Cells

Interferon α/β Sensor Cells

Catalog # hkb-ifnab

For research use only

Version # 16I20-MM

PRODUCT INFORMATION

Contents:

• **1 vial of HEK-Blue™ IFN-α/β 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.

• **100 µl of Blasticidin** (10 mg/ml).

Store blasticidin at 4 °C or at -20 °C.*

• **100 µl Zeocin™** (100 mg/ml).

Store Zeocin™ at 4°C or at -20°C.*

• **1 ml of Normocin™** (50 mg/ml).

Normocin™ is 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 pouch of QUANTI-Blue™** (SEAP detection medium).

Store QUANTI-Blue™ pouch at 4 °C for 12 months. Reconstituted medium is stable at 4 °C for 2 weeks. Protect QUANTI-Blue™ from light.

Handling Cells Upon Receipt

Cells must be thawed **immediately** upon receipt and grown according to handling procedures (as described overleaf), 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™ IFN-α/β cells were stimulated by various cytokines. As expected, only IFN-α or IFN-β induced the production of SEAP.

• These cells are guaranteed mycoplasma-free.

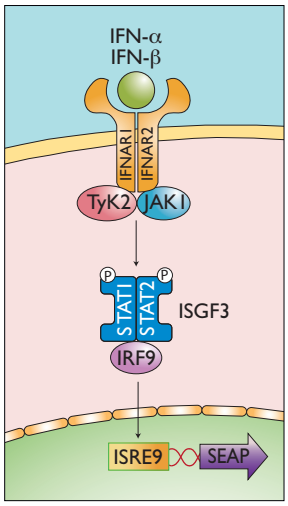
Cell Line Stability

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™ IFN-α/β cells should not be passaged more than 20 times to remain fully efficient. HEK-Blue™ IFN-α/β cells should be maintained in growth medium supplemented with the two selective antibiotics, blasticidin and Zeocin™. Antibiotic pressure with blasticidin is required to maintain the plasmid coding for human STAT2 and IRF9 genes, and Zeocin™ is required to maintain the plasmid coding for SEAP.

INTRODUCTION

Type I interferons, in particular interferon alpha (IFN-α) and interferon beta (IFN-β), play a vital role in host resistance to viral infections. They signal mainly through the JAK-STAT pathway. Following their production, IFN-α and IFN-β bind to a common receptor (IFNAR) and recruit the Janus kinases (JAK1 and TyK2). Jaks phosphorylate STAT1 and STAT2, which then dimerize and interact with IFN regulatory factor 9 (IRF9), forming a complex named ISGF3. ISGF3 binds to IFN-stimulated response elements (ISRE) in the promoters of IFN-stimulated genes (ISG) to regulate their expression.



CELL LINE DESCRIPTION

HEK-Blue™ IFN-α/β cells are specifically designed to monitor the activation of the JAK-STAT pathway induced by type I IFNs. These cells were generated by stably introducing the human STAT2 and IRF9 genes into HEK293 cells to obtain a fully active type I IFN signaling pathway. The other genes of the pathway (IFNAR1, IFNAR2, JAK1, TyK2 and STAT1) are naturally expressed in sufficient amounts. The activation of this pathway is made detectable by the addition of a reporter gene expressing a secreted embryonic alkaline phosphatase (SEAP) under the control of the ISG54 promoter. ISG54 is a well-known ISG activated through an ISRE-dependent mechanism by type I IFNs.

HEK-Blue™ IFN-α/β cells are resistant to the selectable markers blasticidin and Zeocin™. Upon IFN-α or IFN-β stimulation, HEK-Blue™ IFN-α/β cells activate the JAK-STAT pathway and subsequently the expression of the reporter gene. SEAP which is secreted in the supernatant is easily detectable when using QUANTI-Blue™, a SEAP detection reagent.

USE RESTRICTIONS

These cells are distributed for research purposes only.

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SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ IFN-α/β 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, 10% (v/v) fetal bovine serum, 50 U/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml Normocin™, 2 mM L-glutamine

• Freezing Medium: DMEM with 20% fetal bovine serum and 10% (v/v) DMSO

• Test Medium: DMEM, 4.5 g/l glucose, 10% (v/v) heat-inactivated fetal bovine serum (30 min at 56°C), 50 U/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml Normocin™, 2 mM L-glutamine

Note: Heat-inactivated FBS is also commercially available.

Required Selective Antibiotic(s)

• Blasticidin and Zeocin™.

Frozen cells

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 (approximately 2 minutes).

2- Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol.

Note: All of the operations 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 selective 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 selective antibiotics.

6- Transfer the vial contents to a T-25 tissue culture flask containing 5 ml of growth medium without selective antibiotics.

7- Place the culture at 37°C in 5% CO_2.

Cell maintenance

- Maintain and subculture the cells in growth medium supplemented with 30 µg/ml of blasticidin and 100 µg/ml of Zeocin™.
- Renew growth medium twice a week.
- Cells should be passaged when a 70-80% confluency is reached. Do not let the cell grow to 100% confluency.

Interferon α/β Detection

Day 1:

- Add 20 µl of each sample per well of a flat-bottom 96-well plate.
- Add 20 µl of IFN-α/β at 10^4 U/ml stock solution (positive control) in one well.
- Add 20 µl of IFN-γ at 10^4 U/ml (negative control) in one well.
- Prepare a cell suspension of HEK-Blue™ IFN-α/β cells at ~280,000 cells per ml in test medium (containing 10% v/v heat-inactivated FBS).

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.

- Add 180 µl of cell suspension (~50,000 cells) per well.
- Incubate the plate at 37°C in a CO_2 incubator for 20-24 h.

Day 2:

- Prepare QUANTI-Blue™ following the instructions on the enclosed product data sheet.
- Add 180 µl of resuspended QUANTI-Blue™ per well of a flat-bottom 96-well plate.
- Add 20 µl of induced HEK-Blue™ IFN-α/β cells supernatant.
- Incubate the plate at 37°C incubator for 1-3 h.
- Determine SEAP levels using a spectrophotometer at 620-655 nm.

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RELATED PRODUCTS

Product	Description	Cat.Code
Blasticidin	Selective antibiotic	ant-bl-1
QUANTI-Blue™	SEAP detection reagent	rep-qb1
Zeocin™	Selective antibiotic	ant-zn-1

TECHNICAL SUPPORT
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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.

	96-well plate	24-well plate	12-well plate
QUANTI-Blue™	200 µl	500 µl	1 ml
Supernatant	20/5 µl	50/10/5 µl	100/25/10 µl

RELATED PRODUCTS

Product	Catalog Code
pNiFty2-SEAP (Zeo^R)	pnifty2-seap
pSELECT-zeo-SEAP	psetz-seap
Recombinant SEAP Protein	rec-hseap

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