HEK-Blue™ TNF-α Cells

TNF-α Sensor Cells

Catalog # hkb-tnfdmyd

For research use only

Version # 15K26-MM

PRODUCT INFORMATION

Contents

- **1 vial of HEK-Blue™ TNF-α 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 Zeocin™** (100 mg/ml).

Store Zeocin™ at 4°C for 6 months or at -20°C for long term storage.

- **100 µl Puromycin** (10 mg/ml).

Store puromycin at 4°C for 6 months or at -20°C for long term storage.

- **1 ml Normocin™** (50 mg/ml).

Normocin™ is a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20°C. Product is stable for 18 months when stored at -20°C.

- **1 pouch QUANTI-Blue™** (SEAP detection reagent).

Store QUANTI-Blue™ pouch at room temperature for up to 6 months. Reconstituted QUANTI-Blue™ medium is stable 2 weeks at 4°C. Keep away 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.

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™ TNF-α cells should not be passaged more than 20 times to remain fully efficient. HEK-Blue™ TNF-α Cells should be maintained in Growth Medium supplemented with the selective antibiotic, Zeocin™ (100 µg/ml). Antibiotic pressure with Zeocin™ is required to maintain the plasmid coding for SEAP.

Quality Control

Reporter activity is validated by stimulating the cells with human and murine TNF-α.

The cells are guaranteed mycoplasma-free.

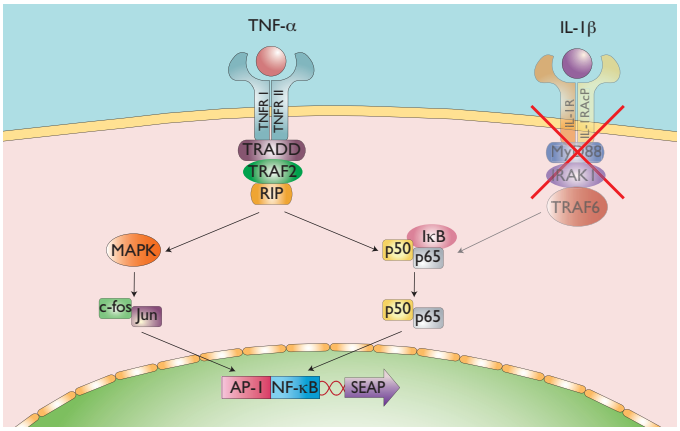
USE RESTRICTIONS

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 the terms and conditions of all applicable Limited Use Label Licenses.

BACKGROUND

Tumor necrosis factor-alpha (TNF-α) is a pleiotropic inflammatory cytokine produced by several types of cells, predominantly activated macrophages. TNF-α plays an important role in the immune response to microbial invasions and in the necrosis of specific tumors. TNF-α binds two receptors TNFR1 and TNFR2 inducing a signaling that involves TRADD, TRAF2 and RIP, and leads to the activation of the NF-κB and the MAPK pathways. interleukin 1 beta (IL-1β) is another inflammatory cytokine that triggers these pathways following the binding to its receptor IL-1RI and the recruitment of MyD88. Both TNF-α and IL-1β receptors are expressed in HEK293 cells.



CELL LINE DESCRIPTION

HEK-Blue™ TNF-α cells allow the detection of bioactive human and murine TNF-α by monitoring the activation of the NF-κB pathway. These cells were generated by stable transfection of HEK293 cells with a SEAP reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB (and five AP-1) binding sites. They were further rendered unresponsive to IL-1β by knocking-out the MyD88 gene.

Stimulation of HEK-Blue™ TNF-α cells with TNF-α triggers the activation of the NF-κB-inducible promoter and the production of SEAP. Levels of SEAP in the supernatant can be easily determined using QUANTI-Blue™, a reagent that turns purple/blue in the presence of SEAP, by reading the optical density (OD) at 620-655 nm.

HEK-Blue™ TNF-α cells are resistant to Zeocin™ and puromycin.

SAFETY CONSIDERATIONS

HEK-Blue™ TNF-α 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.

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HANDLING PROCEDURES

Required Cell Culture Medium

• **Growth Medium:** DMEM, 4.5 g/l glucose, 10% fetal bovine serum, 2 mM L-glutamine, 100 µg/ml Normocin™
Note: Heat-inactivated FBS is also commercially available.
• **Freezing Medium:** DMEM, 4.5 g/l glucose, 20% fetal bovine serum, 10% DMSO
• **Test Medium:** DMEM, 4.5 g/l glucose, 10% heat-inactivated FBS (30 min at 56 ºC), 2 mM L-glutamine, 100 µg/ml Normocin™
Note: Heat-inactivated FBS is also commercially available.

Required Selective Antibiotic(s)

• Zeocin™
• Puromycin

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.

- 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).
- 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.
- 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.**
- Centrifuge vial at 1000-1200 RPM (RCF = 200-300 g) for 5 minutes.
- Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium without selective antibiotics.
- Transfer the vial contents to a T-25 culture flask containing 5 ml of growth medium without selective antibiotics.
- Place the culture at 37 °C in 5% CO_2.

Frozen Stock Preparation

- Resuspend cells at a density of 3-7 x 10^6 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.
- Aliquot 1 ml cells into cryogenic vials.
- Place vials in a freezing container and store at -80 ºC overnight.
- Transfer vials to liquid nitrogen for long term storage.
Note: If properly stored, cells should remain stable for years.

Cell maintenance

- Maintain and subculture the cells in growth medium supplemented with 100 µg/ml of Zeocin™ and 1 µg/ml of puromycin.
Note: We recommend the use of Pen-Strep (50 U/ml-50 µg/ml) together with Normocin™ to keep the cells free of microbial contaminants.
- 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.
Note: Use HEK-Blue™ TNF-α cells with less than 20 passages.

DETECTION OF TNF-α

Sample preparation

- Warm the samples at 37 ºC before use.
Note: Make sure that your samples do not contain alkaline phosphatase activity as it may interfere with the SEAP detetion assay.

Cell handling procedure

To ensure the best results:
- Use a culture maintained around 6.10^5 cells/ml or 70 to 80% confluence.

Day 1:

- Remove medium and rinse with PBS
- Detach the cells from the flask with PBS (7 ml of PBS for a 75 cm^2 tissue culture flask) and homogenize the cell suspension by gentle pipetting.
- Resuspend cells in fresh test medium and prepare a cell suspension at ~280,000 cells/ml.
- Add 20 µl of sample per well of a flat-bottom 96-well plate including the positive (recombinant TNF-α) and negative (test medium or sterile PBS) controls.
4- Add 180 µl of cell suspension (~50,000 cells) per well.
5- Incubate the plate at 37 °C in a 5% CO_2 incubator for 18-24 h.

Day 2:

- Prepare QUANTI-Blue™ following the instructions on the enclosed product data sheet.
- Add 180 µl of QUANTI-Blue™ per well of a flat-bottom 96-well plate.
- Add 20 µl of induced HEK-Blue™ TNF-α cells supernatant.
- Incubate the plate at 37 °C incubator for 30 min to 3 h.
- Determine SEAP levels using a spectrophotometer at 620-655 nm.

EC50 Values of HEK-Blue™ TNF-α cells for TNF-α

The EC50 values of HEK-Blue™ TNF-α cells for human and mouse TNF-α were determined using commercially available cytokines diluted in Test Medium or water.
• **hTNF-α EC50:** 0.01 ng/ml (in medium) or 0.7 ng/ml (in water)
• **mTNF-α EC50:** 0.1 ng/ml (in medium) or 3 ng/ml (in water)

RELATED PRODUCTS

Product	Catalog Code
Anti-hTNF-α-IgG1 antibody	htnfa-mab1
Normocin™	ant-nr-1
QUANTI-Blue™	rep-qb1
Recombinant human TNF-α1a	htnf-a1a
Zeocin™	ant-zn-1

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