

HEK-Blue™ hNOD2 Cells

SEAP Reporter 293 cells expressing the human NOD2 gene

Catalog code: hkb-hnod2

<https://www.invivogen.com/hek-blue-hnod2>

For research use only

Version 23E15-MM

PRODUCT INFORMATION

Contents and Storage

• 3-7 x 10⁶ of HEK-Blue™ hNOD2 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 pouch of HEK-Blue™ Detection, a cell culture medium (50 ml) for real-time detection of SEAP. Store pouch at 4°C for 6 months. Reconstituted HEK-Blue™ Detection is stable for 2 weeks at 4 °C. Protect from light.

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 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 various pathogen recognition receptor (PRR) agonists has been validated using functional assays. As expected, NOD2 agonists induced the production of SEAP.
- The expression of the human NOD2 gene has been confirmed by RT-PCR.
- 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 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.

PRODUCT DESCRIPTION

HEK-Blue™ hNOD2 cells are designed for studying the stimulation of human NOD2 (hNOD2) by monitoring the activation of NF-κB. HEK-Blue™ hNOD2 cells were obtained by co-transfection of the hNOD2 gene and an optimized secreted embryonic alkaline phosphatase (SEAP) reporter gene into HEK293 cells. The SEAP reporter gene is placed under the control of the IL-12 p40 minimal promoter fused to five NF-κB and AP-1 binding sites. Stimulation with a NOD2 ligand activates NF-κB and AP-1 which induce the production of SEAP.

Levels of SEAP can be easily determined with HEK-Blue™ Detection, a cell culture medium that allows for real-time detection of SEAP. HEK-Blue™ Detection is a one-step procedure and extremely simple to use. It is applicable to high-throughput screening. HEK-Blue™ Detection contains all the nutrients necessary for cell growth and a specific SEAP color substrate. The hydrolysis of the substrate by SEAP produces a purple/blue color that can be easily detected with the naked eye or measured with a spectrophotometer.

SEAP activity can also be assessed using the alkaline phosphatase detection reagent, QUANTI-Blue™ Solution. With the QUANTI-Blue™ assay, cells are stimulated in a culture medium containing heat-inactivated fetal bovine serum. Following cell activation, QUANTI-Blue™ Solution is used to detect SEAP in the cell supernatant. This colorimetric assay allows the same cell cultures to be repeatedly sampled for kinetic studies or further experimentation.

For more information, visit <https://www.invivogen.com/quanti-blue>.

HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1.

Note: The parental cell line for HEK-Blue™ hNOD2 cells is HEK-Blue™ Null2 cells (SEAP reporter cells which do not express hNOD2).

SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ hNOD2 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.

TECHNICAL SUPPORT

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

 **InvivoGen**
www.invivogen.com

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), Pen-Strep (100 U/ml-100 µg/ml), 100 µg/ml **Normocin™**
- **Freezing Medium:** DMEM with 20% FBS and 10% (v/v) DMSO

Required Selective 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 selective antibiotics until the cells have been passaged twice.**
4. Centrifuge vial at 300 x g (RCF) 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 25 cm² tissue culture flask containing 5 ml of growth medium without selective antibiotics.
7. Place the culture at 37 °C in 5% CO₂.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10⁶ 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. 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.
Note: If properly stored, cells should remain stable for years.

Cell maintenance

1. HEK-Blue™ hNOD2 cells grow as adherent cells. Detach the cells using trypsin for 2-3 min at room temperature (RT).
Note: The response of HEK-Blue™ hNOD2 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 mins.
2. Maintain and subculture the cells in growth medium supplemented with 30 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.
Note: The average doubling time for the HEK-Blue™ hNOD2 cells is ~24 hours using the conditions described above.

NOD2 Stimulation determined using HEK-Blue™ Detection

HEK-Blue™ Detection is a cell culture medium that allows the detection of SEAP as the reporter protein is secreted by the cells. Prepare HEK-Blue™ Detection following the instructions on the enclosed data sheet.

Note: Before the test, the cells should be 50-80% confluent.

1. Add 20 µl of each sample per well of a flat-bottom 96-well plate.
2. Add 20 µl of a positive control (such as **L18-MDP**, 100 ng/ml) in one well.
3. Add 20 µl of a negative control (such as sterile, endotoxin-free water) in one well.
4. Remove HEK-Blue™ hNOD2 cells from the incubator and discard growth medium.
5. Gently rinse cells with pre-warmed 5-10 ml PBS (for a T-75 flask).
6. Add 2-5 ml pre-warmed PBS (for a T-75 flask) and place the cells at 37 °C for 1- 2 min, detach the cells by tapping the flask. Dissociate cell clumps by gently pipetting up and down.
Note: For the reporter assay, do not use trypsin to detach HEK-Blue™ hNOD2 cells.
7. Count cells which have been resuspended in pre-warmed PBS.
Note: For the reporter assay, avoid centrifugation of HEK-Blue™ hNOD2 cells.
8. Prepare a cell suspension ~280,000 cells per ml in **HEK-Blue™ Detection** medium and immediately add 180 µl of the cell suspension (~50,000 cells) per well.
Note: Avoid prolonged incubation of cells at room temperature in **HEK-Blue™ Detection** medium as it may lead to high background or false positive readings.
9. Incubate the plate at 37 °C in 5% CO₂ for 16-24 h. SEAP can be observed with naked eye and determined using a spectrophotometer at 620-655 nm.

Specificity of HEK-Blue™ hNOD2 Cells

As HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1, HEK-Blue™ hNOD2 cells will respond to their cognate ligands, such as **poly(I:C)**, **flagellin** and **C12-iE-DAP**, respectively. In order to identify NOD2-specific responses, we recommend to use **HEK-Blue™ Null2** cells as a control cell line.

Note: HEK-Blue™ hNOD2 cells may be stimulated in a NOD2-independent manner as NF-κB/AP-1 can be activated by a wide variety of stimuli (e.g. TNF-α and PMA).

RELATED PRODUCTS

Product	Description	Catalog Code
Blasticidin	Selection antibiotic	ant-bl-1
HEK-Blue™ Detection	SEAP detection medium	hb-det2
HEK-Blue™ Null2 Cells	Parental Cells	hkb-null2
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
L18-MDP	NOD2 ligand	tlrl-lmdp
MDP	NOD2 ligand; L-D isoform)	tlrl-mdp
MDP Control	Inactive D-D isoform	tlrl-mdpc
N-Glycolyl-MDP	NOD2 ligand	tlrl-gmdp
Normocin™	Antimicrobial reagent	ant-nr-1
Zeocin®	Selection antibiotic	ant-zn-1

TECHNICAL SUPPORT

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