HEK-Dual™ hTLR3 Cells

Human TLR3-expressing NF-κB & IRF reporter HEK293 cells

Catalog code: hkd-htlr3

https://www.invivogen.com/hek-dual-htlr3

For research use only

Version 23H25-AK

PRODUCT INFORMATION

Content

- **3-7 x 10^6 of HEK-Dual™ hTLR3 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 Hygromycin B Gold** (100 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 3 antibiotics active against mycoplasmas, bacteria and fungi. Store at -20 °C.*

*The expiry date is specified on the product label.

- **1 tube of QUANTI-Luc™ 4 Reagent**, a lucia luciferase detection reagent (sufficient to prepare 25 ml). Store at -20 °C. Avoid repeated freeze-thaw cycles. *Note: This product is photosensitive and should be protected from light.*
- **1 ml of QB reagent** and **1 ml of QB buffer** (sufficient to prepare 100 ml of **QUANTI-Blue™ Solution**, a SEAP detection reagent). Store QB reagent and QB buffer 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

Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Cells will undergo genotypic changes resulting in reduced responsiveness over time in normal cell culture conditions. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages. To ensure maximum efficiency, do not passage HEK-Dual™ hTLR3 cells more than 20 times.

Quality Control

- Human TLR3 expression has been verified by RT-qPCR and functional assays.
- 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 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 info@invivogen.com.

PRODUCT DESCRIPTION

HEK-Dual™ hTLR3 cells are designed to monitor the human Toll-like receptor 3 (hTLR3)-dependent NF-κB and IRF reponses in HEK293 cells.

These cells were generated from the HEK-Dual™ cell line through the stable expression of hTLR3. They feature two reporter genes allowing the simultaneous study of the IRF pathway, by monitoring the activity of an inducible secreted Lucia luciferase and the NF-κB pathway by monitoring the activity of an inducible SEAP (secreted embryonic alkaline phosphatase). Lucia luciferase and SEAP activities are readily assessable in the supernatant using QUANTI-Luc™ and QUANTI-Blue™ Solution, respectively.

HEK-Dual™ hTLR3 cells are responsive to synthetic analogs of dsRNA, in contrast to their parental cell line HEK-Dual™. As expected, these cells show potent NF-κB and IRF responses upon incubation with TLR3-specific ligands, such as Poly(I:C) (polyinosinic-polycytidylic acid) or Poly(A:U) (polyadenylic–polyuridylic acid).

HEK-Dual™ hTLR3 cells are selectable with Blasticidin, Hygromycin, and Zeocin®.

BACKGROUND

The endosomal Toll-like receptor 3 (TLR3) recognizes double-stranded (ds)RNA, a hallmark of viral replication, and triggers potent antiviral immune responses[1]. It is expressed on myeloid dendritic cells, macrophages, as well as non-immune cells[2]. TLR3 activation upon viral infection involves several steps, including translocation of TLR3 from the ER (endoplasmic reticulum) to the endosome, proteolytic cleavage and dimerization, and finally receptor-ligand binding[3]. In order to start the signaling cascade, activated TLR3 recruits the adaptor protein TRIF (TIR domain-containing adapter-inducing interferon-β). Subsequently, TRIF interacts with TRAF3 (TNF receptor-associated factor 3) and TRAF6 to trigger TBK1 (TANK-binding kinase 1)-dependent IRF3 and TRAF6-dependent NF-κB responses, respectively[3]. Given its important role in viral recognition, TLR3 signaling has been intensively studied in the context of antiviral defense, vaccine development and cancer research[4].

1. **Manuela Sironi, *et al.*, 2012.** A Common Polymorphism in TLR3 Confers Natural Resistance to HIV-1 Infection. J Immunol 15; 188 (2): 818–823. 2. **Aluri, J, *et al.*, 2021.** Toll-Like Receptor Signaling in the Establishment and Function of the Immune System. Cells, 10, 1374. 3. **Chen Y, *et al.*, 2021.** Toll-like receptor 3 (TLR3) regulation mechanisms and roles in antiviral innate immune responses. J Zhejiang Univ Sci B.;22(8):609-632. 4. **Komal A, *et al.*, 2021.** TLR3 agonists: RGC100, ARNAX, and poly-IC: a comparative review. Immunol Res. 69(4):312-322.

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com

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

InvivoGen
www.invivogen.com

SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Dual™ hTLR3 cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) and thus may require Biosafety Level 2. The biosafety level varies by country. In the United States, HEK293 cell lines are designated Biosafety Level 2 according to the Center for Disease Control and Prevention (CDC). 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), 100 µg/ml Normocin™, Pen-Strep (100 U/ml-100 µg/ml)
- **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 Blasticidin, Hygromycin B Gold, Zeocin®, andNormocin™.**

Required Selective Antibiotics

Blasticidin, Hygromycin B Gold, 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 into 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 cm^2 tissue culture flask containing 5 ml of growth medium without selective antibiotics.
7. Place the flask containing cells at 37 °C in 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 3-5 x 10^6 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.

Note: If properly stored, cells should remain stable for years.

Cell Maintenance

1. HEK-Dual™ hTLR3 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. After cells have recovered (after at least one passage), subculture the cells in growth medium supplemented with 10 µg/ml of Blasticidin, 100 µg/ml of Hygromycin B Gold, 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.

REPORTER ASSAY PROTOCOL

Below is a protocol using HEK-Dual™ hTLR3 cells together with their parental cell line HEK-Dual™ to monitor the NF-κB and IRF responses. It is recommended to perform the assay with test medium that does **not** contain **Blasticidin, Hygromycin B Gold, Zeocin®, and Normocin™.**

Induction of HEK-Dual™ hTLR3 cells

Day 1

1. Add 20 µl of TLR3 ligand (e.g. Poly(I:C) HMW at 1 µg/ml final concentration) per well of a flat-bottom 96-well plate.
2. Include a NF-κB positive control (e.g. recombinant human (h) TNF-α at 10 ng/ml), an IRF positive control (e.g. recombinant hIFN-β at 1000 U/ml), and a negative control (e.g. endotoxin free water).
3. Detach HEK-Dual™ hTLR3 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-Dual™ hTLR3 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. Using PBS to detach the cells might increase the background activity of NF-κB and/or IRF.

4. Add 180 µl of the cell suspension (~50.000 cells) per well.
5. Incubate overnight at 37 °C in 5% CO_2.

Day 2

The NF-κB and IRF induction of HEK-Dual™ hTLR3 cells can be detected using the SEAP reporter with QUANTI-Blue™ Solution and the Lucia luciferase reporter with QUANTI-Luc™ 4 Lucia/Gaussia, respectively.

Detection of the NF-κB response

1. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed product data sheet.
2. Add 20 µl of induced cell supernatant.
3. Add 180 µl of resuspended QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
4. Incubate the plate at 37 °C for 30 min to 1 hour.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

Detection of the IRF response

Below is a protocol for end-point readings using a luminometer with an injector. This protocol can be adapted for use with a luminometer with or without an injector for kinetic measurements.

1. Prepare QUANTI-Luc™ 4 Reagent working solution following the instructions on the data sheet.

2. Set the luminometer with the following parameters: 50 µl of injection, end-point measurement with a 4 second start time and 0.1 second reading time.
3. Pipet 10 - 20 µl of cell culture supernatant per well in a 96-well white (opaque) or black plate, or a luminometer tube.
4. Prime the injector with QUANTI-Luc™ 4 Reagent working solution
5. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Cat. Code
HEK-Dual™ cells	hkd-nfis
Poly(I:C) HMW	tlrl-pic
Poly(A:U)	tlrl-pau
QUANTI-Blue™ Solution	rep-qbs
QUANTI-Luc™ 4 Lucia/Gaussia	rep-qlc4lg1
Recombinant hTNF-α	rcyc-htnfa
Blasticidin	ant-bl-1
Hygromycin B Gold	ant-hg-1
Normocin™	ant-nr-1
Zeocin®	ant-zn-1