HepG2-Dual™ Cells

NF-κB-SEAP and IRF-Lucia reporter liver carcinoma cells

Catalog code: hepg2d-nfis

https://www.invivogen.com/hepg2-dual

For research use only

Version 24I12-AK

PRODUCT INFORMATION

Contents and Storage

- **3-7 x 10^6 of HepG2-Dual™ 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 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

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. HepG2-Dual™ cells should not be passaged more than 20 times to remain fully efficient. HepG2-Dual™ cells should be maintained in growth medium supplemented with Blasticidin and Zeocin®.

Quality Control

- Reporter activity has been verified by functional assays.
- The stability for 20 passages following thawing has been verified.
- The cells are guaranteed mycoplasma-free.

USE RESTRICTIONS

These cells are distributed for internal research use for non-profit recipients only.

BACKGROUND

The HepG2 cell line is a human hepatocellular carcinoma that is often used for *in vitro* studies of infection with hepatitis viruses and other hepatotropic pathogens[1,2]. Antiviral immune responses mostly rely on recognition of viral nucleic acids as pathogen-associated molecular patterns (PAMPs) by cytosolic Toll-like receptors (TLRs), cytosolic RIG-I-like receptors (RLRs), and cytosolic DNA sensors (CDS). The TLR expression and activation profile of HepG2 cells is still poorly characterized. On the contrary, it is established that HepG2 cells express the RIG-I and MDA-5 RNA sensors[2,3]. Although they do not express the cyclic GMP-AMP synthase (cGAS) cytosolic DNA sensor, these cells express low levels of its adaptor protein, STING (stimulator of interferon genes, also known as MITA)[4].

1. Gural N. *et al.*, 2017. Engineered livers for infectious diseases. Cell Mol Gastroenterol Hepatol. 5:131-44. **2. Yin X. *et al.*, 2017.** Hepatitis E virus persists in the presence of a type III interferon response PLoS Pathog. 13(5):e1006417. **3. Sato S. *et al.*, 2015.** The RNA sensor RIG-I dually functions as an innate sensor and direct antiviral factor for hepatitis B virus. Immunity. 42(1):123-32. **4. Dansako H. *et al.*, 2016.** The cyclic GMP-AMP synthetase-STING signaling pathway is required for both the innate immune response against HBV and the suppression of HBV assembly. FEBS J. 2283(1):144-56.

CELL LINE DESCRIPTION

HepG2-Dual™ cells derive from the HepG2 liver carcinoma cell line. They stably express a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB binding sites. These cells also express the secreted Lucia luciferase reporter gene under the control of an ISG54 minimal promoter in conjunction with five IFN-stimulated response elements (ISRE) which bind the interferon regulatory factors (IRF). Both reporter proteins are readily measurable in the cell culture supernatant by using QUANTI-Blue™ Solution, a SEAP detection reagent, and QUANTI-Luc™ 4 Lucia/Gaussia, a Lucia and Gaussia luciferase detection reagent. As a result, HepG2-Dual™ cells allow to simultaneously study the NF-κB and IRF pathways by assessing the activity of SEAP and Lucia luciferase, respectively.

HepG2-Dual™ cells were stimulated with numerous ligands. None of the TLR ligands tested induced an NF-κB or IRF response, with the exception of the TLR2 ligand Pam3CSK4, which induced a weak NF-κB response. Consistent with the defective expression of cGAS in the parental cell line, HepG2-Dual™ cells did not respond to VACV-70/LyoVec™ stimulation. A moderate IRF response is detected in HepG2-Dual™ cells upon incubation with STING agonists such as 2'3'-cGAMP, while the 3p-hpRNA RIG-I agonist, an *in vitro* transcribed 5' triphosphate hairpin RNA, induces a strong IRF response. The cytokines IL-1β and TNF-α can be used as positive controls to activate the NF-κB pathway, while type I interferons can be used as positive controls to induce the IRF pathway. HepG2-Dual™ cells are resistant to Blasticidin and Zeocin®.

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

SAFETY CONSIDERATIONS

Biosafety Level 1

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** Eagle's minimal essential medium (EMEM), 10% (v/v) heat-inactivated fetal bovine serum (FBS; 30 min at 56 °C), 1X non-essential amino acids (NEAA), Pen-Strep (100 U/ml-100 µg/ml), 100 µg/ml Normocin™
- **Freezing Medium:** EMEM with 20% (v/v)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% 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 T-25 culture flask containing 5 ml of growth medium.
7. Place the culture 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.

Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.

2. Dispense 1 ml of cell suspension 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. After cells have recovered and are growing well (after at least two passages), maintain and subculture the cells in growth medium supplemented with 10 µg/ml of Blasticidin and 100 µg/ml of Zeocin®.
2. Renew growth medium twice a week.
3. Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency. Rinse cell layer with PBS and detach cells by incubating with 0.25% trypsin-EDTA for 5-10 minutes at 37 °C. Do not use a cell scraper.

*Note: Do **not** expose cells to trypsin for more than 10 minutes.*

4. As HepG2-Dual™ cells easily form clumps, gently dissociate cells using a 5 ml syringe and 18-gauge needle for accurate cell counting.

Note: To ensure the best results:

- *Use HepG2-Dual™ cells with less than 20 passages after thawing.*
- *Handling of cells should be as short as possible to prevent any damage resulting from the prolonged stay at room temperature without 5% CO_2.*

REPORTER ASSAYS

Cell preparation

1. Rinse cell layer with PBS and detach cells with trypsin.
2. Centrifuge cells at 300 x g (RCF) for 5 minutes.
3. Remove supernatant and resuspend cells at 1.1 x 10^5 cells/ml in **test medium** (EMEM, 10% (v/v) heat-inactivated FBS, 1X NEAA, Pen-Strep (100 U/ml-100 µg/ml) **without Normocin™, Blasticidin and Zeocin®.**

NF-κB induction

1. Add 20 µl of test sample per well of a flat-bottom 96-well plate, including a positive control (e.g. recombinant human TNF-α at 100 ng/ml or recombinant human IL-1β at 1 ng/ml final concentration), and endotoxin free water as a negative control

Note: Use new tips for each well to avoid cross-contamination.

2. Add 180 µl of cell suspension (~20,000 cells) per well.
3. Incubate the plate at 37 °C in a CO_2 incubator for 18-24 h.
4. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed data sheet (TDS).
5. Add 20 µl of induced HepG2-Dual™ cell supernatant per well of a new flat-bottom 96-well plate.
6. Add 180 µl of QUANTI-Blue™ Solution.
7. Incubate the plate at 37 °C in a CO_2 incubator for 1-8 h.
8. Determine NF-κB-induced SEAP levels using a microplate spectrophotometer at 620-655 nm.

IRF induction

Below is a protocol for end-point readings using a luminometer. It can be adapted for use with kinetic measurements.

1. Add 20 µl of sample per well of a flat-bottom 96-well plate, including a positive control (e.g. recombinant human IFN-β at 1 x 10^4 IU/ml final concentration) and endotoxin free water as a negative control.

Note: Use new tips for each well to avoid cross-contamination.

2. Add 180 µl of cell suspension (~20,000 cells) per well.
3. Incubate the plate at 37 °C in a CO_2 incubator for 18-24 h.
4. Prepare QUANTI-Luc™ 4 Reagent working solution following the instructions on the enclosed TDS.
5. Transfer 20 µl of stimulated cells supernatant into a 96-well white (opaque) or black plate, or a luminometer tube.
6. Add 50 µl of QUANTI-Luc™ 4 Reagent working solution.
7. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Description	Cat. Code
2'3'-cGAMP	STING ligand	tlrl-nacga23
3p-hpRNA	RIG-I ligand	tlrl-hprna
Blasticidin	Selection antibiotic	ant-bl-1
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs1
QUANTI-Luc™ 4 Lucia/Gaussia	Luminesence detection kit	rep-qlc4lg1
Zeocin®	Selection Antibiotic	ant-zn-1

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InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com