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 23A06-MM

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 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.

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

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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QUANTI-Blue™ Solution

Medium for detection and quantification of alkaline phosphatase in standard and HTS assays

Catalog code: rep-qbs, rep-qbs2, rep-qbs3

https://www.invivogen.com/quanti-blue

For research use only

Version 23A12-MM

PRODUCT INFORMATION

Contents: QUANTI-Blue™ Solution is available in three pack sizes

- **rep-qbs:** 5 x 1 ml of QB reagent and 5 x 1 ml QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **25 x 96-well plates** (500 ml using the standard procedure) or **20 x 1536-well plates** (85 ml using the HTS screening procedure).
- **rep-qbs2:** 10 x 1 ml of QB reagent and 10 x 1 ml QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **50 x 96-well plates** (1 L using the standard procedure) or **40 x 1536-well plates** (170 ml using the HTS screening procedure).
- **rep-qbs3:** 1 x 20 ml bottle of QB reagent and 1 x 20 ml bottle of QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **100 x 96-well plates** (2 L using the standard procedure) or **80 x 1536-well plates** (340 ml using the HTS screening procedure).

Required Material (not provided)

- Sterile water
- Sterile screw cap tube, glass bottle or flask

Storage and stability

- Product is shipped at room temperature. Upon receipt, store QB reagent and QB buffer at -20°C. Product is stable for 1 year at -20°C when properly stored.
- The 20 ml bottles of QB reagent and QB buffer are designed for single use. If required, individual aliquots of QB reagent and QB buffer can be prepared upon receipt or following a single freeze-thaw cycle. Store aliquots at -20°C. **Avoid repeated freeze-thaw cycles**.

Note: During storage, a precipitate may form in the 20 ml bottle of QB reagent. If this occurs, vortex the product until the precipitate disappears. The formation of a precipitate does not affect the activity of the product.

- Reconstituted QUANTI-Blue™ Solution is stable for 2 weeks at 2-8°C and for 2 months at -20°C. Protect QUANTI-Blue™ from light.

Quality Control

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.

- Physicochemical characterization (pH, appearance).
- Functional assays using alkaline phosphatase or SEAP-expressing reporter cells.

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™ Solution changes from pink 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 glycosylphosphatidylinositol (GPI)-anchored protein. SEAP is secreted into the cell culture supernatant and therefore offers many advantages over intracellular reporters.

QUANTI-Blue™ is highly sensitive for quantitative measurement. It has a higher saturation threshold than with pNPP (p-nitrophenyl phosphate) resulting in more significant differences between no, low or high AP activity. Another advantage of QUANTI-Blue™ is that it can determine secreted AP activity without disturbing cells, thus allowing the repeated sampling of cell cultures for kinetic studies.

METHODS

QUANTI-Blue™ Solution has been optimized for use in 96-well plates (standard procedure) and in 1536-well plates (high throughput screening procedure).

A. Standard procedure

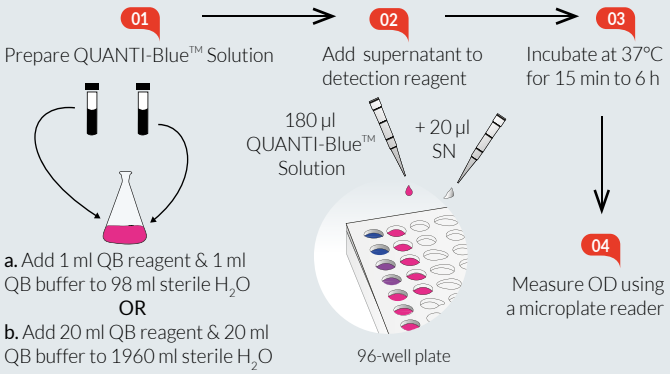

Figure 1. Standard procedure using QUANTI-Blue™ Solution.

The following protocol refers to the use of 96-well plates. Ensure QB reagent and QB buffer are completely thawed before use.

*Note: For fast thawing, QB reagent and QB buffer can be placed at 37°C for 2 minutes. Ensure heating at 37°C does **not** exceed 5 minutes.*

1. In a sterile bottle or flask, prepare QUANTI-Blue™ Solution by adding:
   a. **1 ml** of QB reagent and **1 ml** of QB buffer to **98 ml** of sterile water.
   b. **20 ml** of QB reagent and **20 ml** of QB buffer to **1960 ml** of sterile water.
2. Mix by vortexing and incubate at room temperature for 10 min before use.
3. Use QUANTI-Blue™ Solution immediately or store at 2-8°C or -20°C.
4. Dispense 180 µl of QUANTI-Blue™ Solution per well into a flat-bottom 96-well plate.
5. Add 20 µl of the sample (supernatant of SEAP-expressing cells) or negative control (cell culture medium).
6. Incubate at 37°C for 15 min to 6 h.
7. Measure optical density (OD) at 620-655 nm using a microplate reader.

Note: If the negative control turns purple/blue, it means the fetal bovine serum (FBS) contains alkaline phosphatase. We recommend heating FBS at 56°C for 30 min to inactivate the alkaline phosphatase activity.

For different cell culture plate formats, please refer to the table below:

	96-well plate	24-well plate	12-well plate
QUANTI-Blue™	180 µl	450 µl	900 µl
Supernatant	20 µl	50 µl	100 µl

TECHNICAL SUPPORT
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B. High Throughput Screening (HTS) procedure

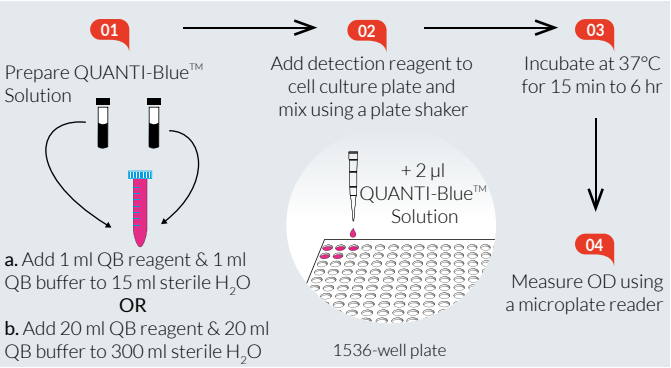

Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

This procedure has been optimized for use in HTS screening procedures in 1536-well plates. QUANTI-Blue™ Solution is added directly to the cell suspension to reduce liquid handling.
Ensure QB reagent and QB buffer are completely thawed before use.
Note: For fast thawing, QB reagent and QB buffer can be placed at 37 °C for 2 minutes. Ensure heating at 37 °C does ***not*** *exceed 5 minutes.*

1. Dispense cell suspension and test compounds into a 1536-well plate in a volume that does not exceed **5 µl** per well. Incubate cells with test compounds for the desired period of time.
2. Prepare QUANTI-Blue™ Solution by adding:
   a. **1 ml** of QB reagent and **1 ml** of QB buffer to **15 ml** of sterile water in a sterile 50 ml screw cap tube.
   b. **20 ml** of QB reagent and **20 ml** of QB buffer to **300 ml** of sterile water in a sterile glass bottle or flask.
3. Mix well by vortexing and incubate at room temperature for 10 minutes before use.
4. Use QUANTI-Blue™ Solution immediately or store at 2-8 °C or -20 °C.
5. Dispense **2 µl of QUANTI-Blue™ Solution** to the wells containing ≤ 5 µl of cell culture in a 1536-well plate.
6. Mix using a plate shaker.
7. Incubate at 37 °C for 15 min to 6 h.
8. Measure OD at 620-655 nm.

Note: If the negative control turns purple/blue, it means the fetal bovine serum (FBS) contains alkaline phosphatase. We recommend heating FBS at 56 °C for 30 min to inactivate the alkaline phosphatase activity.

RELATED PRODUCTS

Product	Catalog Code
pNiFty2-SEAP (Zeo®)	pnifty2-seap
pSELECT-zeo-SEAP	psetz-seap
HEK-Blue™ Detection	hb-det2
Recombinant SEAP Protein	rec-hseap
Reporter cells	
HEK-Blue™ hTLR2	hkb-htlr2
HEK-Blue™ hTLR4	hkb-htlr4
RAW-Blue™ Cells	raw-sp
THP1-Blue™ NF-κB Cells	thp-nfkb
THP1-Blue™ ISG Cells	thp-isg

For a complete list of InvivoGen's Reporter Cell Lines visit
https://www.invivogen.com/reporter-cells

QUANTI-Luc™ 4 Reagent

A coelenterazine-based luminescence assay reagent

https://www.invivogen.com/quanti-luc

For research use only

Version 23A16-MM

PRODUCT INFORMATION

Contents

- 1 tube of **QUANTI-Luc™ 4 Reagent (20X)**

One tube of QUANTI-Luc™ 4 Reagent is sufficient for 5 x 96-well plates (25 ml standard Flash/end-point detection).

Note: This sample cannot be sold separately from the QUANTI-Luc™ 4 Lucia/Gaussia kit.

QUANTI-Luc™ 4 Lucia/Gaussia comprises two liquid components:

- QUANTI-Luc™ 4 Reagent 20X (coelenterazine substrate)
- QUANTI-Luc™ 4 Stabilizer 25X (optimized Glow assay reagent)

Find more information at https://www.invivogen.com/quanti-luc.

Storage and Stability

– Store QUANTI-Luc™ 4 Reagent at -20°C for up to 12 months.
– After preparation, the working solution is stable for 48 hours at 4°C and 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.
Note: This product is photosensitive and should be protected from light.

Quality Control

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.
– Physicochemical characterization (pH, appearance).
– Functional assays using recombinant Lucia protein or reporter cells.

DESCRIPTION

QUANTI-Luc™ 4 Reagent is a component of the QUANTI-Luc™ 4 Lucia/Gaussia kit. It contains the coelenterazine substrate for the detection of secreted Lucia or Gaussia activity in live-cell supernatants, and of intracellular Renilla after cell lysis. The light signal produced correlates to the amount of luciferase protein expressed. It is quantified using a luminometer and expressed as relative light units (RLUs).

METHODS

Preparation of QUANTI-Luc™ 4 Reagent working solution (1X):

1. Dilute the total volume of the 20X tube (1.25 ml) of Reagent into 23.75 ml of sterile water to obtain 25 ml of working solution.
2. Vortex **very briefly** (a few seconds).
3. Use the working solution immediately or store until required for use. QUANTI-Luc™ 4 Reagent working solution can be stored for 48 hours at 4°C or 1 month at -20°C.

Flash detection of luciferase activity from cell culture medium:

To obtain **end-point readings** using a luminometer **with an injector**.
1. 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.
2. Pipet 20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Prime the injector with QUANTI-Luc™ 4 Reagent 1X and proceed **immediately** with the measurement.

To obtain **end-point readings** using a luminometer **without injectors**.
1. Set the luminometer with a 0.1 second reading time.
2. Pipet 20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Add 50 µl of QUANTI-Luc™ 4 Reagent 1X to each well or tube.
4. Gently tap the plate several times to mix (do **not** vortex).
5. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Cat. Code
QUANTI-Luc™ 4 Lucia/Gaussia Kit	
500 tests	rep-qlc4lg1
2 x 500 tests	rep-qlc4lg2
5 x 500 tests	rep-qlc4lg5

TECHNICAL SUPPORT
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