HepG2-Dual™ cells
NF-κB-SEAP & IRF-Lucia reporter liver carcinoma cells
Catalog code: hepg2d-nfis
http://www.invivogen.com/hepg2-dual
For research use only
Version 18L21-MM

PRODUCT INFORMATION
Contents and Storage
• 1 vial of HepG2-Dual™ cells (3-7 x 10⁶ cells)

IMPORTANT: Cells are shipped frozen. If cells 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 QUANTI-Luc™ (Lucia luciferase detection reagent). Store pouch at -20°C. Reconstituted QUANTI-Luc™ is stable for 1 week at 4°C and for 1 month at -20°C. Protect 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.

Handling 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.

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.
• HepG2-Dual™ 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. 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. 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).


CELL LINE DESCRIPTION
HepG2-Dual™ cells derive from the HepG2 liver carcinoma cell line. HepG2-Dual™ cells 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. They 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™, a Lucia 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™.
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) fetal bovine serum 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 1000-1200 RPM (RCF = 200-300 g) 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% CO2.

Frozen Stock Preparation
1. Resuspend cells at a density of 3-5 x 10⁶ 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% CO2.

REPORTER ASSAYS
Cell preparation
1. Rinse cell layer with PBS and detach cells with trypsin.
2. Centrifuge cells at 1000-1500 RPM (RCF 200-300 g) for 5 min.
3. Remove supernatant and resuspend cells at 1.1 x 10⁴ 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-kB 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 CO2 incubator for 18-24 h.
4. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed data sheet.
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 CO2 incubator for 1-8 h.
8. Determine NF-kB-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 10⁴ 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 CO2 incubator for 18-24 h.
4. Prepare the QUANTI-Luc™ assay solution following the instructions on the enclosed data sheet.
5. Transfer 20 μl of HepG2-Dual™ stimulated cells supernatant into a 96-well white (opaque) or black plate, or a luminometer tube.
6. Add 50 μl of QUANTI-Luc™
7. Proceed immediately with the measurement.

RELATED PRODUCTS

<table>
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<tr>
<th>Product</th>
<th>Description</th>
<th>Catalog Code</th>
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<td>tlrl-nacga23</td>
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<td>3p-hpRNA</td>
<td>RIG-I ligand</td>
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<td>5′ppp-dsRNA/LyoVec™</td>
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<td>LyoVec™</td>
<td>Cationic lipid</td>
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<td>QUANTI-Blue™ Solution</td>
<td>SEAP detection medium</td>
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<td>QUANTI-Luc</td>
<td>Lucia detection medium</td>
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<td>Blasticidin</td>
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<td>Zeocin™</td>
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PRODUCT INFORMATION

Contents
QUANTI-Luc™ is provided as packs of individually sealed pouches.
• rep-qlc1: 2 pouches of QUANTI-Luc™
• rep-qlc2: 5 pouches of QUANTI-Luc™
Each pouch contains everything needed to prepare 25 ml of reagent allowing the preparation of 500 wells of a 96-well plate.

Storage and Stability
- Store QUANTI-Luc™ pouches at -20°C for 12 months.
- Reconstituted QUANTI-Luc™ is stable for 1 week at 4°C and for 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.

Note: This product is photosensitive and should be protected from light.

DESCRIPTION
QUANTI-Luc™ is an assay reagent containing all the components required to quantitively measure the activity of Lucia luciferase and other coelenterazine-utilizing luciferases. QUANTI-Luc™ contains the coelenterazine substrate and stabilizing agents for the luciferase reaction. The light signal produced is quantified using a luminometer and expressed as relative light units (RLU). The signal produced correlates to the amount of luciferase protein expressed, indicating promoter activity in the reporter assay.

QUANTI-Luc™ is optimized for use with Lucia luciferase reporter cell lines. Lucia luciferase is a secreted coelenterazine luciferase encoded by a synthetic gene. As Lucia luciferase is secreted, it can be directly measured in the cell culture medium using bioluminescent assays.

InvivoGen provides a recombinant Lucia luciferase protein (see Related Products) which is a positive control for QUANTI-Luc™. A dilution series of the recombinant Lucia luciferase protein can also be used to determine the linear range of the assay.

METHODS
Preparation of QUANTI-Luc™
1. Pour the pouch contents into a 50 ml screw cap tube.
2. Add 25 ml of sterile water.
3. Swirl product gently until powder is completely dissolved.
4. Use QUANTI-Luc™ assay solution immediately or store until required for use. Reconstituted QUANTI-Luc™ can be stored for 1 week at 4°C and for 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.

Note: This product is photosensitive and should be protected from light.

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 10-20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Prime the injector with the QUANTI-Luc™ assay solution 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 10-20 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™ assay solution to each well or tube.
4. Gently tap the plate several times to mix (do not vortex).
5. Proceed immediately with the measurement.

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<th>Product</th>
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<td>psetz-lucia</td>
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<td>Recombinant Lucia™ protein</td>
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<td><strong>Reporter Cells</strong></td>
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<td>THP1-Dual™ (IRF-Lucia/NF-κB-SEAP) Cells</td>
<td>thpd-nfis</td>
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<td>THP1-Lucia™ NF-κB Cells</td>
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For a complete list of InvivoGen’s Lucia luciferase Reporter Cell Lines visit
http://www.invivogen.com/lucia-reporter-cells
QUANTI-Blue™ Solution
Medium for detection and quantification of alkaline phosphatase in standard and HTS assays
Catalog code: rep-qbs, rep-qbs2
http://www.invivogen.com/quanti-blue

For research use only
Version 18D13-MM

PRODUCT INFORMATION
Contents
QUANTI-Blue™ Solution is available in two pack sizes:
  • rep-qbs containing 5 x 1 ml of QB reagent and 5 x 1 ml QB buffer to prepare 500 ml of QUANTI-Blue™ Solution sufficient for 25 x 96-well plates (standard procedure) or 20 x 1536-well plates (HTS screening)
  • rep-qbs2 containing 10 x 1 ml of QB reagent and 10 x 1 ml QB buffer to prepare 1 liter of QUANTI-Blue™ Solution sufficient for 50 x 96-well plates (standard procedure) or 40 x 1536-well plates (HTS screening)

Required Material (not provided)
  • Sterile water
  • Sterile screw cap tube, glass bottle or flask

Storage and Stability
  • Store QB reagent and QB buffer at -20°C. Product is stable for 1 year at -20°C when properly stored.
  • Reconstituted QUANTI-Blue™ Solution is stable for 2 weeks at 2-8°C and for 2 months at -20°C. Keep reconstituted QUANTI-Blue™ away from light.

Quality Control
Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.
  • Physicochemical characterization (including pH, solubility).
  • 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 GPI-anchored protein. SEAP is secreted into cell culture supernatant and therefore offers many advantages over intracellular reporters.

FEATURES AND ADVANTAGES
  • Requires small samples of cell supernatants - 20 µl is sufficient.
  • No need to process samples - Preparation of cell lysates or heating of samples is not required.
  • Determine secreted AP activity without disturbing cells - The same cell cultures can be repeatedly sampled for kinetic studies.
  • Assay can be completed in 30 min - Hands-on time no longer than 10 min. The enzymatic activity can be detected as early as 15 min after incubation of the samples in QUANTI-Blue™ Solution.
  • Wide dynamic range allows to detect low and high levels of AP - No need to perform multiple sample dilutions.
  • Highly sensitive for quantitative measurement - Higher saturation threshold than with pNPP (p-nitrophenyl phosphate) resulting in more significant differences between no, low or high AP activity.
  • Extremely simple to use - 1) Prepare solution with water, 2) add sample to detection reagent, 3) incubate at 37°C, and 4) assess AP activity.

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.

1. Prepare 100 ml of QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml of QB buffer to 98 ml of sterile H2O.
2. Mix well 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. Keep reconstituted QUANTI-Blue™ away from light.
4. Dispense 180 µl of QUANTI-Blue™ Solution per well into a flat-bottom 96-well plate.
5. Add 20 µl of 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 to heat 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:

<table>
<thead>
<tr>
<th></th>
<th>96-well plate</th>
<th>24-well plate</th>
<th>12-well plate</th>
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<tbody>
<tr>
<td>QUANTI-Blue™</td>
<td>180 µl</td>
<td>450 µl</td>
<td>900 µl</td>
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<tr>
<td>Supernatant</td>
<td>20 µl</td>
<td>50 µl</td>
<td>100 µl</td>
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B. High Throughput Screening procedure

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

This procedure has been optimized for use directly in flat-bottom 1536-well plates, in which cell culture volume does not exceed 5 µl. 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. Prepare 17 ml of QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml of QB buffer to 15 ml of sterile H2O in a 50 ml screw cap tube.
2. Mix well by vortexing and incubate at room temperature for 10 minutes before use.
3. Use QUANTI-Blue™ Solution immediately or store at 2-8°C or -20°C.
4. Dispense 2 µl of QUANTI-Blue™ Solution per well of a 1536-well plate.
5. Mix using a plate shaker.
6. Incubate at 37°C for 15 min to 6 h.
7. Measure 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 to heat FBS at 56°C for 30 min to inactivate the alkaline phosphatase activity.

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<thead>
<tr>
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For a complete list of InvivoGen’s Reporter Cell Lines visit http://www.invivogen.com/reporter-cells