HepG2-Lucia™ AhR cells

AhR hepatoma reporter cells

Catalog code: hpgl-ahr

https://www.invivogen.com/hepg2-lucia-ahr

For research use only

Version 23A06-MM

PRODUCT INFORMATION

Contents and Storage

- **3-7 x 10^6 of HepG2-Lucia™ AhR 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 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.*

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

Quality Control

- Reporter activity has been verified by functional assays.
- The stability for 20 passages following thawing has been verified.
- HepG2-Lucia™ AhR cells are guaranteed mycoplasma-free.

USE RESTRICTIONS

These cells are distributed for research purposes only.

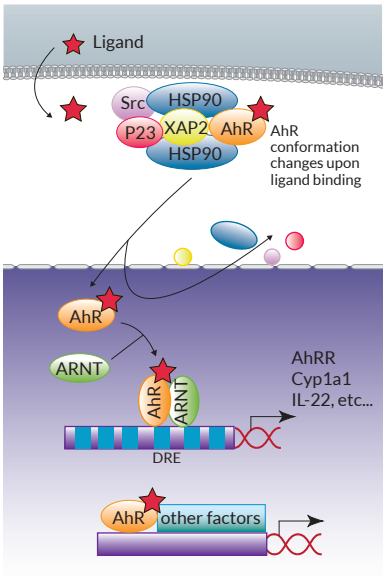
This product is covered by a Limited Use License. By use of this product, the buyer agrees to the terms and conditions of all applicable Limited Use Label Licenses. For non-research use, such as screening, quality control or clinical development, contact info@invivogen.com.

REFERENCES

1. Lamas B. *et al.* 2018. Aryl hydrocarbon receptor and intestinal immunity. Mucosal Immunol. 11:1024-38. **2. Gao J. *et al.* 2018.** Impact of the gut microbiota on intestinal immunity mediated by tryptophan metabolism. Front. Cell. Infect. Microbiol. 8:13. **3. Iwanari M. *et al.*, 2002.** Induction of CYP1A1, CYP1A2, and CYP1B1 mRNAs by nitropolycyclic aromatic hydrocarbons in various human tissue-derived cells: chemical-cytochrome P450 isoform-, and cell-specific differences. Arch. Toxicol. 76:287-98.

BACKGROUND

The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcriptional factor widely expressed in barrier tissues[1]. AhR plays a key role in gut-microbiota and host's immune homeostasis, not only in the intestine but also at distant sites[1]. Besides xenobiotics, including the prototypic AhR agonist 2,3,7,8-tetrachlorodibenzo -p-dioxin (TCDD), a variety of dietary-derived AhR ligands have been identified, many of which are byproducts of tryptophan (Trp) metabolism[2]. Inactive AhR resides in the cytoplasm within a Hsp90:XAP2:p23:Src protein complex. The AhR canonical genomic signaling pathway occurs as follows: upon ligand binding, the complex undergoes conformational changes and translocates into the nucleus. AhR heterodimerizes with AhR nuclear translocator (ARNT) before binding to dioxin response elements (DREs) in the upstream regulatory regions of AhR target genes, such as the cytochrome P450-dependent monooxygenase Cyp1a1, the AhR repressor (AhRR), and the IL-22 interleukin. Of note, non-canonical AhR signaling pathways have also been reported, either at the genomic level through association with other transcription factors (e.g. NF-κB), or at the non-genomic level (e.g. through the release of the Src kinase)[2].



CELL LINE DESCRIPTION

HepG2-Lucia™ AhR cells are engineered from the human HepG2 hepatoma cell line, which expresses endogenous AhR and is of great interest for the detection/screening of AhR ligands in food or environmental samples[3]. HepG2-Lucia™ AhR cells stably express the secreted Lucia luciferase reporter gene under the control of a minimal promoter coupled with the human Cyp1a1 gene entire regulatory sequence, which contains six DREs. The Lucia luciferase reporter protein is readily measurable in the cell culture supernatant by using QUANTI-Luc™ 4 Lucia/Gaussia. HepG2-Lucia™ AhR cells respond to xenobiotics such as TCDD. Additionally, these cells respond to Trp-derived compounds such as indole-3-acetic acid (IAA), 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl esther (ITE), 6-formylindolo[3,2-b]carbazole (FICZ) and L-Kynurenine. Of note, InvivoGen's AhR agonists do not induce activation of the interferon regulatory factors nor of the NF-κB transcription factor (as tested on our HepG2-Dual™ cells). HepG2-Lucia™ AhR cells are resistant to Zeocin®.

TECHNICAL SUPPORT

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

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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

- 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 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-Lucia™ AhR cells easily form clumps, gently dissociate cells using a 5 ml syringe and 18-gauge needle for accurate cell counting.

Notes: To ensure the best results

- Use HepG2-Lucia™ AhR 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™ and Zeocin®.

AhR 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. FICZ at 1 µg/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 24-48 h.
4. Prepare QUANTI-Luc™ 4 Reagent working solution following the instructions on the enclosed data sheet.
5. Transfer 20 µl of HepG2-Lucia™ AhR 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
CH-223191	AhR inhibitor	inh-ch22
FICZ	AhR ligand	tlrl-ficz
HT29-Lucia™ AhR Cells	AhR reporter cells	ht2l-ahr
QUANTI-Luc™ 4 Lucia/Gaussia	Luminesence detection kit	rep-qlc4lg1
Zeocin®	Selection Antibiotic	ant-zn-1

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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