HT29-Lucia™ AhR cells

AhR colorectal adenocarcinoma reporter cells

Catalog code: ht2l-ahr

https://www.invivogen.com/ht29-lucia-ahr

For research use only

Version 19B25-MM

PRODUCT INFORMATION

Contents and Storage

- **1 vial of HT29-Lucia™ AhR cells** (3-7 x 10^6 cells)

IMPORTANT: Cells are shipped frozen. If cells 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 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.

Note: Data sheets for all components are available on our website.

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. HT29-Lucia™ AhR cells should not be passaged more than 20 times to remain fully efficient. HT29-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.
- HT29-Lucia™ AhR cells are guaranteed mycoplasma-free.

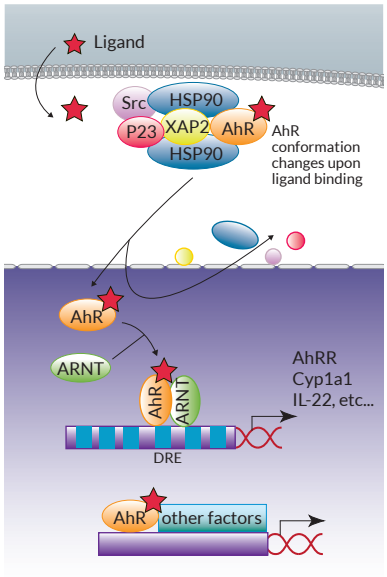
USE RESTRICTIONS

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.

BACKGROUND

The aryl hydrocarborn 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)[2,3].



CELL LINE DESCRIPTION

HT29-Lucia™ AhR cells are engineered from the human HT-29 colorectal adenocarcinoma cell line which expresses endogenous AhR[3]. Their anatomical origin and ability to differentiate into intestinal enterocyte-like cells and mucus-producing cells make these cells highly relevant for studying intestinal microbiota-related ligands for AhR[4]. Furthermore, HT29-Lucia™ AhR cell-based assay is an adequate model to study AhR signaling in intestinal cancerous cells. HT29-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

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in the cell culture supernatant by using QUANTI-Luc™. HT29-Lucia™ AhR cells respond to TCDD, as well as 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 Plus. Importantly, in HT29-Lucia™ AhR cells, Lucia luciferase is not induced by other pattern recognition receptor ligands.
HT29-Lucia™ AhR cells are resistant to Zeocin™.

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. Park JH. *et al.*, 2018.** Kynurenine promotes the goblet cell differentiation of HT-29 colon carcinoma cells by modulating Wnt, Notch and AhR signals. Oncol Rep. 39(4):1930-8. **4. Martínez-Maqueda D. *et al.* 2015.** HT29 Cell Line. Bioactives on Health: in vitro and ex vivo models. Springer. Chapt 1.

SAFETY CONSIDERATIONS

Biosafety Level 1

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, 4.5 g/l glucose, 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 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% 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: To ensure the best results:
- Use 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 1000-1200 RPM (RCF 200-300 g) for 5 min.
3. Remove supernatant and resuspend cells at 2.8 x 10^5 cells/ml in **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 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 5 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 (~50,000 cells) per well.
3. Incubate the plate at 37 °C in a CO_2 incubator for 24-48 h.
4. Prepare the QUANTI-Luc™ assay solution following the instructions on the enclosed data sheet.
5. Transfer 20 µl of HT29-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™
7. Proceed **immediately** with the measurement.

Alternatively, Lucia luciferase activity can be detected using **QUANTI-Luc™ Gold**, a reporter reagent kit with two protocols for either enhanced luminescent signal or enhanced signal stability (ideal for high-throughput screening or time-course studies).

RELATED PRODUCTS

Product	Description	Catalog Code
CH-223191	AhR inhibitor	inh-ch22
FICZ	AhR ligand	tlrl-ficz
HepG2-Lucia™ AhR Cells	AhR reporter cells	hpgl-ahr
ITE	AhR ligand	tlrl-ite
L-Kynurenine	AhR ligand	tlrl-kyn
QUANTI-Luc™	Lucia detection medium	rep-qlc1
QUANTI-Luc™ Gold	For standard and HTS assays	rep-qlcg1
Zeocin™	Selection antibiotic	ant-zn-1

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QUANTI-Luc™

A coelenterazine-based luminescence assay reagent

Catalog code: rep-qlc1, rep-qlc2

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

For research use only

Version 18D30-MM

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.

RELATED PRODUCTS

Product	Catalog Code
QUANTI-Luc™ Gold (For standard and HTS assays)	rep-qlcg1
pSelect-zeo-Lucia™ (expression plasmid)	psetz-lucia
Recombinant Lucia™ protein	rec-lucia
Reporter Cells	
THP1-Dual™ (IRF-Lucia/NF-κB-SEAP) Cells	thpd-nfis
THP1-Lucia™ NF-κB Cells	thp1-nfkb

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

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