HEK-Lucia™ RIG-I Cells

Lucia luciferase reporter HEK293 cells expressing human RIG-I

Catalog code: hkl-hrigi

https://www.invivogen.com/hek-lucia-rigi

For research use only

Version 23A06-MM

PRODUCT INFORMATION

Contents

- **3-7 x 10^6 of HEK-Lucia™ RIG-I 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 3 antibiotics active against mycoplasma, 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 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

Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Cells will undergo genotypic changes resulting in reduced responsiveness over time in normal cell culture conditions. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages. To ensure maximum efficiency, do not passage HEK-Lucia™ RIG-I cells more than 20 times. These cells should be maintained in growth medium supplemented with selective antibiotics, Blasticidin (30 µg/ml) and Zeocin® (100 µg/ml).

Quality Control

- The expression of the human RIG-I (hRIG-I) gene has been confirmed by qRT-PCR.
- The reporter activity has been validated by stimulating these cells with various RIG-I agonists.
- The cell line stability for 20 passages following thawing has been verified.
- HEK-Lucia™ RIG-I cells are guaranteed mycoplasma-free.

BACKGROUND

RIG-I (retinoic-acid-inducible protein 1, also known as Ddx58) receptor is a cytoplasmic RNA helicase that is critical for host antiviral responses. It senses double-stranded RNA (dsRNA), a replication intermediate for RNA viruses, leading to production of type I interferons (IFNs) in infected cells[1]. Notably, RIG-I detects viral RNAs that exhibit an uncapped 5'-di/triphosphate end and a short blunt-ended double stranded (ds) portion[2].

Upon activation, it is recruited by the adaptor MAVS (also known as IPS-1, CARDIF or VISA) to the outer membrane of the mitochondria leading to the activation of several transcription factors including interferon-regulatory factor 3 (IRF3), IRF7 and NF-κB[3]. Activated IRF3 and IRF7 bind to IFN-stimulated response elements (ISRE) in the promoters of IFN-stimulated genes (ISG) leading to a type I IFN-mediated immune response.

1. Gebhardt A. *et al.*, 2017. Discrimination of Self and Non-Self Ribonucleic Acids. Journal of Interferon & Cytokine Research 37: 184-97. 2. Pichlmair A. *et al.*, 2006. RIG-I-mediated antiviral responses to single-stranded RNA bearing 5'-phosphates. Science 314:997-1001. 3. Kawai T. *et al.*, 2005. IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. Nat Immunol. 6(10):981-988.

CELL LINE DESCRIPTION

HEK-Lucia™ RIG-I cells were generated from HEK-Lucia™ Null cells, HEK293-derived cells that stably express the secreted Lucia luciferase reporter gene. This reporter gene is under the control of an IFN-inducible ISG54 promoter enhanced by a multimeric IFN-stimulated response elements (ISRE). HEK-Lucia™ RIG-I cells stably express high levels of human RIG-I and respond strongly to cytosolic RIG-I ligands such as 3p-hpRNA and 5'ppp-dsRNA (see validation sheet).

HEK-Lucia™ RIG-I and HEK-Lucia™ Null cells can be used to study the role of RIG-I by monitoring IRF-induced Lucia luciferase activity. The levels of IRF-induced Lucia in the cell culture supernatant can be easily monitored using QUANTI-Luc™ 4 Lucia/Gaussia, a Lucia and Gaussia luciferase detection reagent.

Note: HEK-Lucia™ RIG-I and HEK-Lucia™ Null cells endogenously express NOD1, TLR3 and TLR5.

HEK-Lucia™ RIG-I cells are resistant to blasticidin, G418, hygromycin and Zeocin®. They should be maintained in growth medium (see next page) supplemented with blasticidin and Zeocin®.

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.

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 2

HEK-Lucia™ RIG-I cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) and thus may require Biosafety Level 2. The biosafety level varies by country. In the United States, HEK293 cell lines are designated Biosafety Level 2 according to the Center for Disease Control and Prevention (CDC). In Germany, HEK293 cell lines are designated Biosafety Level 1 according to the Central Committee of Biological Safety, Zentrale Kommission für die Biologische Sicherheit (ZKBS). Please check with your country's regulatory authority regarding the use of these cells.

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% 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% FBS, 10% DMSO
- **Test Medium:** DMEM 4.5 g/l glucose, 2 mM L-glutamine, 10% heat-inactivated FBS, Pen-Strep (100 U/ml-100 µg/ml) **without Normocin™, Blasticidin, and Zeocin®**

Required Selective Antibiotics

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% (v/v) ethanol.

Note: All steps from this point should be carried out under strict aseptic conditions.

3. Transfer cells into 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 min.
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 25 cm^2 tissue culture flask containing 5 ml of growth medium without selective antibiotics.
7. Place the flask containing cells at 37 °C in 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10^6 cells/ml in freezing medium freshly prepared with cold growth medium.

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

2. Dispense 1 ml of the 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 (after at least one passage), subculture the cells in growth medium supplemented with 30 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 cell grow to 100% confluency.

Induction of HEK-Lucia™ RIG-I Cells

Day 1:

1. Add 20 µl of each sample per well of a flat-bottom 96-well plate.
2. Add 20 µl of a positive control such as cytosolic 3p-hpRNA at 100 ng/ml in one well.

Note: To achieve 3p-hpRNA stimulation of RIG-I, 3p-hpRNA must be delivered to the cytoplasm, for example by using a transfection agent, such as LyoVec™.

3. Add 20 µl of a negative control such as sterile, endotoxin-free water in another well.
4. Prepare a cell suspension of HEK-Lucia™ RIG-I cells at ~280,000 cells per ml in test medium.
5. Add 180 µl of cell suspension (~50,000 cells) per well.
6. Incubate the plate at 37 °C in a CO_2 incubator for 20-24 h.

Reporter Assay

Below is a protocol for end-point readings using a luminometer and QUANTI-Luc™ 4 Reagent, this protocol can be adapted for use with kinetic measurements.

Day 2:

1. Prepare QUANTI-Luc™ 4 Reagent working solution following the instructions on the enclosed TDS.
2. Pipet 10 µl of HEK-Lucia™ RIG-I cell culture supernatant per well in a 96-well white (opaque) or black plate, or a luminometer tube.
3. Add 50 µl of QUANTI-Luc™ 4 Reagent working solution per well.
4. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Description	Cat. Code
3p-hpRNA	RIG-I ligand	tlrl-hprna
5'ppp-dsRNA	RIG-I ligand	tlrl-3prna
Blasticidin	Selection antibiotic	ant-bl-05
HEK-Lucia™ Null Cells	Parental cell line	hkl-null
LyoVec™	Transfection reagent	lyec-12
Normocin™	Antimicrobial reagent	ant-nr-1
QUANTI-Luc™ 4 Lucia/Gaussia	Luminescence 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
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