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 19F05-MM

PRODUCT INFORMATION

Contents

- **1 vial of HEK-Lucia™ RIG-I Cells** (3-7 x 10^6 cells)
- **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 pouch of QUANTI-Luc™** (Lucia luciferase detection medium)

Store QUANTI-Luc™ pouch at -20 °C for 12 months. Reconstituted QUANTI-Luc™ medium is stable for 1 week at 4 °C and for 1 month at -20 °C. Protect QUANTI-Luc™ from light.

Handling Cells Upon Receipt

Cells must be thawed **immediately** upon receipt and grown according to handling procedures (as described on the next page), to ensure cell viability and proper assay performance.

*Note: **Do not freeze the 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

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.

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

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™, a Lucia 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™.

TECHNICAL SUPPORT
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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 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 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™, this protocol can be adapted for use with kinetic measurements.

Day 2:

1. Prepare QUANTI-Luc™ 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™ 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™	Lucia detection reagent	rep-qlc1
QUANTI-Luc™ Gold	Lucia detection reagent	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

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

For research use only

Version 19A04-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 luciferase 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 https://www.invivogen.com/lucia-reporter-cells.

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