

# HEK-Lucia™ Null Cells

Lucia luciferase reporter HEK293 cells

Catalog code hkl-null

<https://www.invivogen.com/hek-lucia-null>

For research use only

Version 23A06-MM

## PRODUCT INFORMATION

### Contents

• **3-7 x 10<sup>6</sup> of HEK-Lucia™ Null 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 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™ Null cells more than 20 times. These cells should be maintained in growth medium supplemented with the selective antibiotic Zeocin® (100 µg/ml).

### Quality Control

- The reporter activity has been validated by stimulating these cells with type I interferons (IFNs).
- The stability for 20 passages following thawing has been verified.
- HEK-Lucia™ Null 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](mailto:info@invivogen.com).

## CELL LINE DESCRIPTION

HEK-Lucia™ Null cells are HEK293-derived cells that stably express the Lucia luciferase secreted reporter gene. This reporter gene is under the control of the interferon (IFN)-inducible promoter which is comprised of the IFN-stimulated genes (ISG) 54 promoter enhanced by a multimeric IFN-stimulated response elements (ISRE).

HEK-Lucia™ Null cells respond to human type I IFNs. The levels of IFN-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™ Null cells endogenously express NOD1, TLR3 and TLR5.*

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

## SAFETY CONSIDERATIONS

### Biosafety Level 2

HEK-Lucia™ Null 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™ and Zeocin®**

### Required Selective Antibiotics

Zeocin®

## TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

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### 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<sup>2</sup> tissue culture flask containing 5 ml of growth medium without selective antibiotics.
7. Place the flask containing cells at 37 °C in 5% CO<sub>2</sub>.

### Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10<sup>6</sup> 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 100 µg/ml of Zeocin<sup>®</sup>.
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™ Null 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 human IFN-β at 1x10<sup>4</sup> IU/ml in one well.
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™ Null 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<sub>2</sub> 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™ Null 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
HEK-Lucia™ RIG-I Cells	RIG-I reporter cells	hkd-hrigi
Normocin™	Antimicrobial reagent	ant-nr-1
QUANTI-Luc™ 4 Lucia/Gaussia Zeocin <sup>®</sup>	Luminescence detection kit Selection antibiotic	rep-qlc4lg1 ant-zn-1

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# 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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