RAW-Lucia™ ISG-KO-IRF3 Cells

IRF3 knockout ISRE-inducible Lucia luciferase reporter mouse macrophages

Catalog code: rawl-koirf3

https://www.invivogen.com/raw-lucia-isg-ko-irf3

For research use only

Version 23A06-MM

PRODUCT INFORMATION

Contents and Storage

- **1 vial of RAW-Lucia™ ISG-KO-IRF3 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 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.

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

- **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 of 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 cells more than 20 times and maintain cells in growth medium supplemented with the selective antibiotic.

Quality Control

- IRF3 knockout is verified by functional assays and DNA sequencing.
- The stability for 20 passages following thawing has been verified.
- The cells are guaranteed mycoplasma-free.

USE RESTRICTIONS

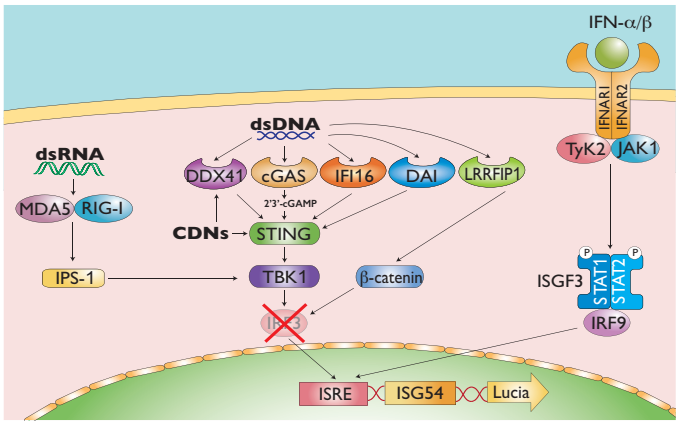
These cells are distributed for research purposes only.

This product is covered by a Limited Use License. By use of this product, the buyer agrees 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.

INTRODUCTION

Interferon regulatory factor 3 (IRF3) is a key transcription regulator of type I interferon (IFN)-dependent innate immunity The presence viral, bacterial, or self nucleic acids, such as dsDNA, dsRNA, or cyclic dinucleotides (CDNs), in the cytoplasm of mammalian cells triggers IFN production. Cytosolic DNA is detected by cytosolic DNA sensors (CDSs), such as cGAS, leading to IFN production through the STING-TBK1-IRF3 pathway[1]. Viral dsRNA is detected by cytoplasmic RNA helicases RIG-I and MDA-5, which interact with the IPS-1 adaptor protein and trigger IFN production through TBK1-IRF3 signaling. CDNs bind directly to STING leading to IRF3-mediated IFN production[2]. Indeed, IRF3 is crucial for the immune response, as it regulates the activation of IFN-stimulated response elements (ISRE) in the promoters of IFN-stimulated genes (ISG).

1. Sun L. *et al.*, 2013. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science 339(6121):786-91. **2. Burdette DL. *et al.*, 2011.** STING is a direct innate immune sensor of cyclic di-GMP. Nature 478(7370):515-8. **3. Lam E. et al., 2014.** Adenovirus Detection by the cGAS/STING/TBK1 DNA Sensing Cascade. J Virol. 88(2):974-81.



PRODUCT DESCRIPTION

RAW-Lucia™ ISG-KO-IRF3 cells were generated from the RAW-Lucia™ ISG cell line, which is derived from the murine RAW 264.7 macrophage cell line, which has been reported to express several CDSs, including cGAS[3]. RAW-Lucia™ ISG and RAW-Lucia™ ISG-KO-IRF3 cells express a secreted reporter gene, Lucia luciferase, under the control of the I-ISG54 promoter which is comprised of the IFN-inducible ISG54 promoter enhanced by a multimeric ISRE. Thus, these cells allow the monitoring of ISRE activation by determining the activity of Lucia luciferase. The levels of ISRE-induced Lucia in the cell culture supernatant can be easily monitored using QUANTI-Luc™ 4 Lucia/Gaussia detection reagent. RAW-Lucia™ ISG-KO-IRF3 cells respond to murine type I IFNs through the JAK-STAT-IRF9 pathway but do not respond to cytosolic nucleic acids, such as poly(dA:dT)/LyoVec™, VACV70/LyoVec™, poly(I:C)/LyoVec™ and 2'3'-cGAMP.

RAW-Lucia™ ISG-KO-IRF3 cells are resistant to Zeocin®.

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

SAFETY CONSIDERATIONS

Biosafety Level 2

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, 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 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 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 150 x g (RCF) for 10 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 5-7 x 10^6 cells/ml in freezing medium freshly prepared with cold DMEM.
   Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.
2. Transfer 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, subculture the cells in growth medium with an initial seeding density of 1.5 x 10^4 cells per cm^2 (e.g. ~1 x 10^6 cells in a T-75 culture flask). To maintain selection pressure, add 200 µg/ml of Zeocin® to the growth medium every other passage.
2. Renew growth medium twice a week.
3. Using a cell scraper, cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

Note: Do **not** *use trypsin.*

Cell-Handling Recommendations

To ensure the best results:

- Use RAW-Lucia™ ISG-KO-IRF3 cells with less than 20 passages.
- Handling of cells should be as short as possible to prevent any damage resulting from a prolonged stay at room temperature without 5% CO_2.

REPORTER ASSAY

Cell Preparation

Pass cells **either** 3 or 4 days prior to the reporter assay.

- If three days, seed cells at a cell density of 2.5 x 10^4 per cm^2 corresponding to ~ 2 x 10^6 cells in a T-75 culture flask.
- If four days, seed cells at a cell density of 1.5 x 10^4 per cm^2 corresponding to ~ 1 x 10^6 cells in a T-75 culture flask.

IRF Induction

1. Remove medium from RAW-Lucia™ ISG-KO-IRF3 cells and rinse twice with warm PBS.
2. Use a cell scraper to detach cells and resuspend cells in test medium which contains 10% (v/v) heat-inactivated FBS and prepare a cell suspension at ~550,000 cells/ml.
3. Add 20 µl of your sample per well of a flat-bottom 96-well plate.
4. Add 20 µl of a positive control (e.g. murine Type I IFN) in another well.
5. Add 20 µl of a negative control (e.g. test medium) in another well.
6. Add 180 µl of cell suspension (~**100,000 cells**) per well.

IMPORTANT: To ensure reliable and reproducible results, make sure homogenize the cell suspension before the cell distribution.

7. Incubate the plate at 37°C in a 5% CO_2 incubator for 18-24 h.
8. Prepare QUANTI-Luc™ 4 Reagent working solution following the instructions on the data sheet.
9. 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.
10. Add 10 µl of RAW-Lucia™ ISG-KO-IRF3 cell culture supernatant per well in a 96-well white (opaque) or black plate, or a luminometer tube.
11. Prime the injector with QUANTI-Luc™ 4 Reagent working solution and proceed with the measurement.

RELATED PRODUCTS

Product	Description	Cat. Code
2'3'-cGAMP	STING ligand	tlrl-nacga23
Normocin™	Antimicrobial Reagent	ant-nr-1
QUANTI-Luc™ 4 Lucia/Gaussia	Luminesence detection kit	rep-qlc4lg1
RAW-Lucia™ ISG	Parental cells	rawl-isg
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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