RAW-Lucia™ ISG-KO-IRF7 Cells

IRF7 knockout IRF-inducible Lucia luciferase reporter murine macrophages

Catalog code: rawl-koirf7

https://www.invivogen.com/raw-lucia-isg-ko-irf7

For research use only

Version 19K14-MM

PRODUCT INFORMATION

Contents and Storage

- **1 vial of RAW-Lucia™ ISG-KO-IRF7 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 Normocin™** (50 mg/ml), a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20 °C.
- **1 pouch of QUANTI-Luc™**. Store QUANTI-Luc™ pouch at -20 °C for 12 months. Reconstituted QUANTI-Luc™ medium is stable 1 week at 4 °C and 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 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

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

- Biallelic IRF7 knockout has been verified by functional assays and DNA sequencing.
- The stability of this cell line for 20 passages following thawing has been verified.
- The cells are guaranteed mycoplasma-free.

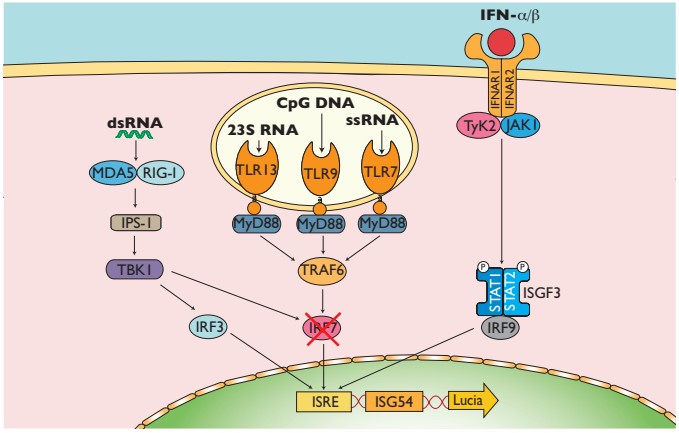
USE RESTRICTIONS

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 7 (IRF7) is a key transcription regulator of type I interferon (IFN)-dependent innate immunity and plays a critical role in the innate immune response against DNA and RNA viruses. IRF7 is constitutively expressed at low levels in the cytoplasm, and becomes activated by innate receptor signaling, resulting in translocation to the nucleus and induction of type I IFN genes and IFN-stimulated genes (ISG) by binding to an interferon-stimulated response element (ISRE) in their promoters. Interestingly, IRF7 has been shown to associate with the constitutively expressed transcription factor IRF3 to induce certain promoters.



PRODUCT DESCRIPTION

RAW-Lucia™ ISG-KO-IRF7 cells were generated from RAW-Lucia™ ISG cells through stable gene knockout of IRF7. These cells derive from the murine RAW 264.7 macrophage cell line, which has been reported to express many pattern recognition receptors (PRRs), including TLR7[1], TLR9[2, 3], and TLR13[4]. The stimulation of these TLRs with their corresponding ligands leads to activation of the IRF-7-dependent type I IFN pathway.

RAW-Lucia™ ISG-KO-IRF7 and RAW-Lucia™ ISG cells can be used to study the role of IRF7 by monitoring of IRF-induced Lucia luciferase activity. They express the gene for secreted Lucia luciferase under the control of ISG54 minimal promoter in conjunction with five ISREs. The levels of IRF-induced Lucia in the cell culture supernatant can be easily monitored using QUANTI-Luc™, a Lucia luciferase detection reagent.

RAW-Lucia™ ISG-KO-IRF7 cells are resistant to Zeocin™.

1. Delgado MA. *et al.*, 2008. Toll-like receptors control autophagy. EMBO J. 27(7):1110-21. **2. Stein SC. *et al.*, 2012.** Cell-specific regulation of nucleic acid sensor cascades: a controlling interest in the antiviral response. J Virol. 86(24):13303-12. **3. West AP. *et al.*, 2011** TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. Nature 472:476–80. **4. Shi Z. *et al.*, 2009.** Transcriptional regulation of the novel Toll-like receptor Tlr13. J Biol Chem. 284(31):20540-7.

TECHNICAL SUPPORT

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

InvivoGen
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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 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 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-IRF7 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-IRF7 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™ 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-IRF7 cell culture supernatant per well in a 96-well white (opaque) or black plate, or a luminometer tube.
11. Prime the injector with the QUANTI-Luc™ assay solution and proceed with the measurement.

RELATED PRODUCTS

Product	Description	Catalog Code
2'3'-cGAMP	STING ligand	tlrl-cga23
3'3'-cGAMP	STING ligand	tlrl-cga
5'ppp-dsRNA	RIG-I ligand	tlrl-3prna
CL264	TLR7 ligand	tlrl-c264e
Gardiquimod™	TLR7 ligand	tlrl-gdqs
HSV-60/LyoVec™	CDS ligand	tlrl-hsv60c
Poly(dA:dT)/LyoVec™	CDS ligand	tlrl-patc
Poly(I:C) (HMW)/LyoVec™	RIG-I ligand	tlrl-piclv
Normocin™	Antimicrobial reagent	ant-nr-1
ODN 1585	TLR9 ligand	tlrl-2395
ODN 1826	TLR9 ligand	tlrl-1826
ODN 2395	TLR9 ligand	tlrl-2395
ORN Sa19	TLR13 ligand	tlrl-orn19
QUANTI-Luc™	Lucia detection reagent	rep-qlc1
QUANTI-Luc™ Gold	Lucia detection reagent	rep-qlcg1
R848	TLR7/8 ligand	tlrl-r848
RAW-Lucia™ ISG-KO-cGAS	cGAS knockout cells	rawl-kocgas
RAW-Lucia™ ISG	Parental cells	rawl-isg
RAW-Lucia™ ISG-KO-IFI16	IFI16 knockout cells	rawl-koif16
RAW-Lucia™ ISG-KO-STING	STING knockout cells	rawl-kostg
VACV-70/LyoVec™	CDS ligand	tlrl-vv70c
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.

TECHNICAL SUPPORT
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