RAW-Lucia™ ISG-KO-IRF5 Cells

IRF5 knockout IRF-inducible Lucia luciferase reporter mouse macrophages

Catalog code: rawl-koirf5

https://www.invivogen.com/raw-lucia-isg-ko-irf5

For research use only

Version 19K14-MM

PRODUCT INFORMATION

Contents and Storage

- **1 vial of RAW-Lucia™ ISG-KO-IRF5 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 mycoplasma, bacteria and fungi. Store at -20 °C.*

*The expiry date is specified on the product label.

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

Handling 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.

Cell Line Stability

Cells will undergo genotypic changes resulting in reduced responsiveness over time in normal cell culture conditions. Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages.

Quality Control

- Biallelic IRF5 knockout has been verified by PCR, DNA sequencing, Western blot, and functional assays.
- The stability for 20 passages, following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

PRODUCT DESCRIPTION

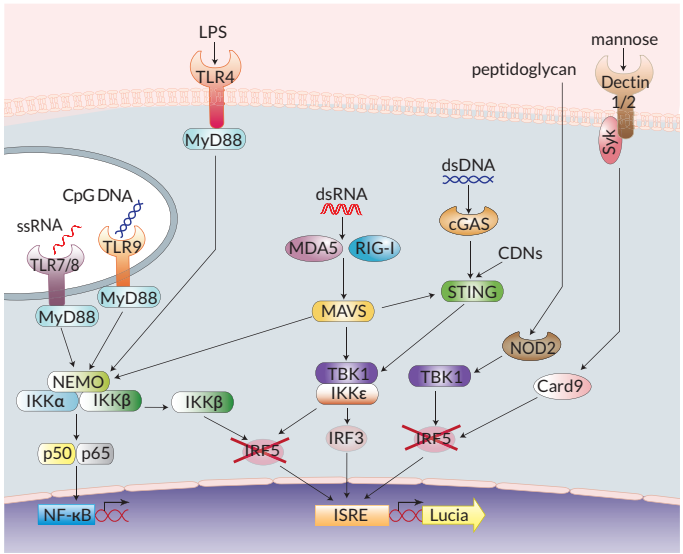
RAW-Lucia™ ISG-KO-IRF5 cells are adherent RAW 264.7-derived murine macrophages that were generated from the RAW-Lucia™ ISG cell line through the stable knockout of *IRF5* gene. These cells feature a Lucia luciferase reporter gene under the control of an ISG54 promoter enhanced by multimeric ISREs. Thus, activation of the IRF pathway can be readily assessed by monitoring the activity of secreted Lucia luciferase in the supernatant using the QUANTI-Luc™ detection reagent. As expected, the IRF response is strongly diminished upon incubation with STING agonists, such as 2'3'-cGAMP. However, different responses are observed when using DNA- or RNA-based agonists with varying transfection reagents. RAW-Lucia™ ISG-KO-IRF5 cells retain full ability to respond to type I interferons (IFN-α and IFN-β) and lipopolysaccharide (LPS).

RAW-Lucia™ ISG-KO-IRF5 cells resistant to Zeocin™.

BACKGROUND

Interferon regulatory factors (IRFs) are transcription factors that play essential roles in immune responses. IRF3, IRF5, and IRF7 are involved in the induction of type I interferon (IFN) downstream of pattern recognition receptors (PRRs), such as the cytosolic DNA/cyclic dinucleotide sensors cGAS/STING, the cytoplasmic RNA sensors, RIG-I and MDA5, Toll-like receptors (TLRs), the NOD-like receptor NOD2, and the C-type lectin receptors Dectin-1 and Dectin-2[1]. Research indicates that, depending on the PRR triggered, IRF5 is activated through different mechanisms[1,2]. For example, nucleic acid sensing by RIG-I, MDA5, cGAS or STING, elicit IRF5 activation through TBK1 (TANK-binding kinase 1). IRF5 can also be activated by the IKKβ kinase downstream of the MAVS adaptor associated with RIG-I or MDA5, or downstream of the MyD88 adaptor associated with TLRs[1]. Activated IRF5 binds to ISRE (IFN-stimulated response elements) in the promoters of ISGs (IFN-stimulated genes). The nature of the PRR ligand and cell type determine the (co-)activation of IRF3, IRF5, and IRF7, and subsequently, the level and subtype of type I IFN[1]. Of note, IRF5 is genetically associated with enhanced production of type I IFNs in systemic lupus erythematosus (SLE)[2]. IRF5 is also a key regulator of macrophage differentiation towards the M1 phenotype (producing IL-12, TNF-α and IFN-γ), thereby influencing downstream adaptive immune responses[2].

1. Zhao G-N. *et al.*, 2015. Interferon regulatory factors: at the crossroads of immunity, metabolism, and disease. Biochim Biophys Acta. 2015 Feb;1852(2):365-78. **2. Jefferies C.A., 2019.** Regulating IRFs in IFN driven disease. Frontiers in immunology. Vol 10. Article 325.



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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.

Cell-Handling Recommendations

To ensure the best results:

- Use RAW-Lucia™ ISG-KO-IRF5 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.

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.*

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-IRF5 cells and rinse twice with warm PBS.
2. Use a cell scraper to detach cells and resuspend cells at ~ 1.1 x 10^6 cells/ml in freshly prepared, pre-warmed test medium.
3. Add 20 µl of test compound 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 (~200,000 cells) per well.

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

7. Incubate the plate at 37°C in a 5% CO_2 incubator for 18-24 h.
8. Prepare QUANTI-Luc™ following instructions on the data sheet.
9. Set the luminometer as follows: 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-IRF5 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.

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 with 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.

RELATED PRODUCTS

Product	Description	Cat. Code
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs1
QUANTI-Luc™	Lucia detection reagent	rep-qlc1
QUANTI-Luc™ Gold	Lucia detection reagent	rep-qlcg1
Normocin™	Antimicrobial agent	ant-nr-1
Zeocin™	Selection antibiotic	ant-zn-1
RAW-Lucia™ ISG Cells	Reporter cell line	rawl-isg
5'ppp-dsRNA	RLR ligand	tlrl-3prna
3p-hpRNA	RLR ligand	tlrl-hprna
G3-YSD	CDS ligand	tlrl-ydna
VACV-70	CDS ligand	tlrl-vav70n
2'3'-cGAMP	STING agonist	tlrl-nacga23

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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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