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 23A06-MM

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10^6 of RAW-Lucia™ ISG-KO-IRF5 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 mycoplasma, 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

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.

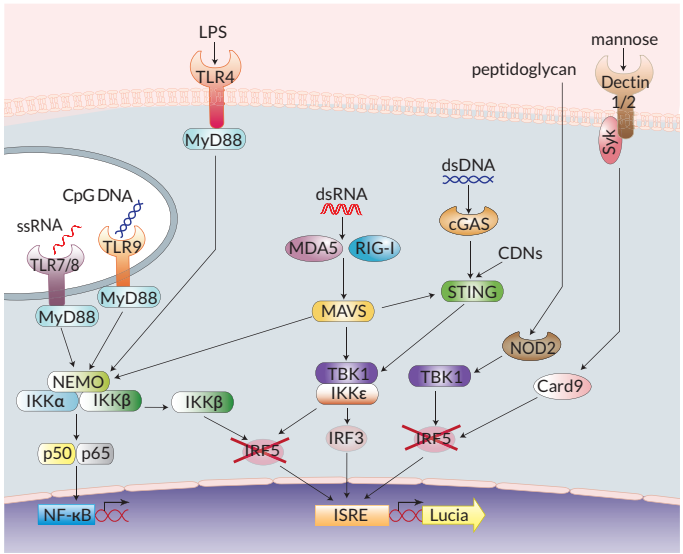
PRODUCT DESCRIPTION

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. Activation of the IRF pathway can be readily assessed by monitoring the activity of secreted Lucia luciferase in the supernatant using the QUANTI-Luc™ 4 Lucia/Gaussia detection reagent. A differential response between RAW-Lucia™ ISG-KO-IRF5 cells and their parental cells is observed when using DNA- or RNA-based agonists with distinct 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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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.

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 growth medium.

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™ 4 Reagent working solution 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 QUANTI-Luc™ 4 Reagent working 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
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

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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

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