RAW-Lucia™ ISG-KO-IRF1 Cells

IRF5 knockout IRF-inducible Lucia luciferase reporter mouse macrophages

Catalog code: rawl-koirf1

https://www.invivogen.com/raw-lucia-isg-ko-irf1

For research use only

Version 23A06-MM

PRODUCT INFORMATION

Contents and Storage

- **3-7 x 10^6 of RAW-Lucia™ ISG-KO-IRF1 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 Normocin™** (50 mg/ml), a formulation of three antibiotics active against mycoplasmas, bacteria, and fungi. Store at -20 °C.*
- **1 ml of Zeocin®** (100 mg/ml). Store at 4°C or 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 over time that will result in reduced responsiveness in normal cell culture conditions. Genetic instability is a biological phenomenon that occurs in all stably transfected cells. It is critical to prepare an adequate number of frozen stocks at early passages.

Quality Control

- Biallelic IRF1 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-IRF1 cells are adherent RAW 264.7-derived murine macrophages that were generated from the RAW-Lucia™ ISG cell line through the stable knockout of the *IRF1* gene. These cells feature a Lucia luciferase reporter gene under the control of an ISG54 promoter enhanced by multimeric ISREs. Thus, activation of IRF pathways can be readily assessed by monitoring the activity of secreted Lucia luciferase in the supernatant using QUANTI-Luc™ 4 Lucia/Gaussia detection reagent. Because IRF1 and other IRF-family members bind to closely related and overlapping ISRE motifs in the ISG54 promoter, RAW-Lucia™ ISG-KO-IRF1 cells display similar induction of the Lucia luciferase reporter when compared to their parental cells.

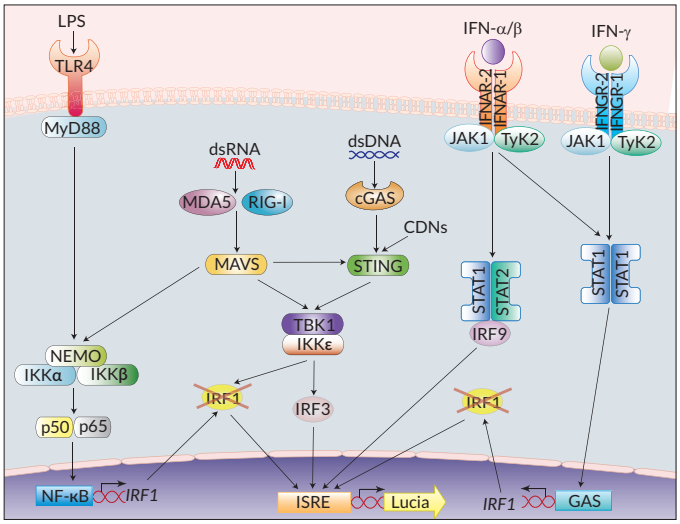
RAW-Lucia™ ISG-KO-IRF1 cells are resistant to Zeocin®.

BACKGROUND

Interferon regulatory factor 1 (IRF1) is the founding member of a family of nine transcription factors (IRF1 to IRF9) playing essential roles in immune responses. IRF1 is expressed at low levels in the cytoplasm of resting cells and is highly induced upon viral infections and inflammatory cytokine signaling. IRF1 dimers translocate to the nucleus and promote the expression of distinct sets of IFN-inducible genes (ISGs) depending on the cell type[1,2]. Notably, IRF1-induced expression upon TLR4 and IFN (mostly IFN-γ) signaling makes it a critical contributor to prolonged waves of ISG expression[2]. IRF1 is also involved in LPS-induced oxidative stress outcomes in macrophages, thus contributing to endotoxemia[3]. Of note, it has been proposed that LPS-mediated IRF1 expression in macrophages controls the production of oxidized mitochondrial DNA, and thus participates in the NLRP3 inflammasome activation[4]. The various biological functions in which IRF1 is involved support the search for IRF1-targeting therapeutic strategies[1-3,5]. Indeed, IRF1 is implicated in many inflammatory diseases, including inflammation-related cancers[2,5,6].

Unlike IRF3, IRF5, and IRF7 which are the principal mediators of IFN induction upon specific PRRs activation, IRF1 rather acts as an amplifier of the gene expression prompted by IRF3, IRF5, or IRF7[7]. Thus, there is no clear phenotype for IRF1-deficient cells.

1. Taniguchi T. *et al.*, 2001. IRF family of transcription factors as regulators of host defense. Annual Rev. Immunol. 19:623. **2. Antonczyk A. *et al.*, 2019.** Direct inhibition of IRF-dependent transcriptional regulatory mechanisms associated with disease. Front. Immunol. 10:1176. **3. Deng S-Y., 2017.** Role of interferon regulatory factor-1 in lipopolysaccharide-induced mitochondrial damage and oxidative stress responses in macrophages. Int. J. Mol. Med. 40:1261. **4. Zhong Z. *et al.*, 2018.** New mitochondrial DNA synthesis enables NLRP3 inflammasome activation. Nature. 560:198. **5. Thompson C.D. *et al.*, 2018.** Therapeutic targeting of IRFs: pathways-dependence or structure-based? Front. Immunol. 9:2622. **6. Hartley G. *et al.*, 2017.** Regulation of PD-L1 expression on murine tumor-associated monocytes and macrophages by locally produced TNF-α. Cancer Immunol. Immunother. 66:523. **7. Jeffries C.A., 2019.** Regulating IRFs in IFN driven disease. Front. Immunol. 10: art325.



TECHNICAL SUPPORT

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

InvivoGen
www.invivogen.com

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, 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 Selection Antibiotics

- 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% (v/v) ethanol.

Note: All steps from this point should be carried out under strict aseptic conditions.

3. Transfer cells to a larger tube containing 15 ml of pre-warmed growth medium. **Do not add selection antibiotics until the cells have been passaged twice.**
4. Centrifuge 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 selection antibiotics.
6. Transfer the cells to a T-25 culture flask with 5 ml of growth medium.
7. Place the culture at 37 °C in 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at 5-7 x 10^6 cells/ml in freshly prepared freezing 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 Handling Recommendations

To ensure the best results:

- Use RAW-Lucia™ ISG-KO-IRF1 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.

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. RAW-Lucia™ ISG-KO-IRF1 cells are adherent. To detach the cells, use a cell scraper when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

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

CELLULAR ASSAYS

The Lucia luciferase activity in the RAW-Lucia™ ISG-KO-IRF1 cells provides a control of IRF induction (including IRF3, IRF5, IRF7, and IRF9) while testing IRF1-dependent phenotypes, such as LPS-induced oxidative stress, or PD-L1 upregulation.

Cell Preparation

Pass cells **either** 3 or 4 days prior to functional assays.

- 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-IRF1 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. 2'3'-cGAMP or 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-IRF1 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.

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 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

TECHNICAL SUPPORT
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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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