RAW-Lucia™ ISG-KO-cGAS Cells

cGAS knockout IRF-inducible Lucia luciferase reporter mouse macrophages

Catalog code: rawl-kocgas

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

For research use only

Version 19K14-MM

PRODUCT INFORMATION

Contents and Storage

- **1 vial of RAW-Lucia™ ISG-KO-cGAS 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 for12 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

- cGAS knockout is 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.

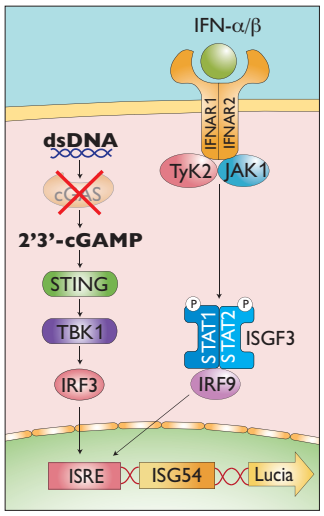
USE RESTRICTIONS

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

Cyclic GMP-AMP synthase (cGAS, cGAMP synthase) is a critical cytosolic DNA sensor that triggers innate immune responses through the production of type I interferons (IFNs)[1]. In response to cytosolic double-stranded DNA (dsDNA), cGAS produces the cyclic dinucleotide (CDN) 2'3'-cGAMP. CDNs bind directly to STING, leading to TBK1-IRF3-mediated activation of IFN-stimulated response elements (ISRE) in the promoters of IFN-stimulated genes (ISG). The most potent agonist of human STING is 2'3'-cGAMP[2,3].



PRODUCT DESCRIPTION

RAW-Lucia™ ISG-KO-cGAS cells were generated from the RAW-Lucia™ ISG cell line, which is derived from the murine RAW 264.7 macrophage cell line, through the stable knockout of the cGAS gene. RAW-Lucia™ ISG-KO-cGAS 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.

RAW 264.7 have been reported to express several cytosolic DNA sensors (CDSs), including cGAS[4]. RAW-Lucia™ ISG-KO-cGAS cells allow the monitoring of interferon regulatory factor (IRF) activation by determining the activity of Lucia luciferase. The levels of IRF-induced Lucia luciferase in the cell culture supernatant can be easily monitored using QUANTI-Luc™, a Lucia luciferase detection reagent.

In this cell line Type I IFNs (IFN-α/β) can be used as positive controls to induce Lucia luciferase through the JAK-STAT-IRF9 pathway.

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

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 Gao P. *et al.*, 2013.** Cyclic [G(2',5')pA(3',5')p] is the metazoan second messenger produced by DNA-activated cyclic GMP-AMP synthase. Cell. 153(5):1094-107. **3. Ablasser A. *et al.*, 2013.** cGAS produces a 2'-5'-linked cyclic dinucleotide second messenger that activates STING. Nature. 498(7454):380-4. **4. Lam E. et al., 2014.** Adenovirus Detection by the cGAS/STING/TBK1 DNA Sensing Cascade. J Virol. 88(2):974-81.

TECHNICAL SUPPORT
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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-cGAS 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-cGAS 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-cGAS 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
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
LyoVec™	Transfection reagent	lyec-12
Normocin™	Antimicrobial reagent	ant-nr-1
QUANTI-Luc™	Lucia detection reagent	rep-qlc1
QUANTI-Luc™ Gold	Lucia detection reagent	rep-qlcg1
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.

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