**RAW-Lucia™ ISG-KO-cGAS Cells**
cGAS knockout IRF-inducible Lucia luciferase reporter mouse macrophages

Catalog # rawl-kogas

For research use only
Version # 15E08-MM

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**PRODUCT INFORMATION**

**Contents and Storage**
- 1 vial of RAW-Lucia™ ISG-KO-cGAS cells (3-7 x 10^6 cells) in freezing medium

**IMPORTANT:** Cells are shipped frozen. If cells are not frozen upon arrival, contact InvivoGen immediately.
- 100 µl Zeocin™ (100 mg/ml). Store Zeocin™ at 4 °C for 6 months, or at -20 °C for long-term storage
- 1 ml Normocin™ (50 mg/ml). Normocin™ is 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 for 1 week at 4 °C and for 1 month at -20 °C. Protect QUANTI-Luc™ from light.

**Handling Cells Upon Arrival**
Cells must be thawed immediately upon receipt and grown according to handling procedures to ensure the best cell viability and assay performance. If you are unable to thaw the cells immediately, frozen cells may be placed in liquid nitrogen until you are ready to thaw and propagate them, however, this may reduce cell viability.

**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.

RAW-Lucia™ ISG-KO-cGAS cells should not be passaged more than 20 times to remain fully efficient. RAW-Lucia™ ISG-KO-cGAS cells should be maintained in growth medium supplemented with the selective antibiotic, Zeocin™ (200 µg/ml). Antibiotic pressure with Zeocin™ is required to maintain the plasmid coding for Lucia luciferase.

**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.

**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.

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**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). 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^1^.

**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 (CDs), including cGAS. 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™.


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**TECHNICAL SUPPORT**
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www.invivogen.com
SAFETY CONSIDERATIONS
Biosafety Level 2

HANDLING PROCEDURES

Required Cell Culture Medium
- Growth Medium: DMEM, 4.5 g/l glucose, 10% fetal bovine serum (FBS), 100 µg/ml Normocin™, 2 mM L-glutamine
- Freezing Medium: DMEM, 4.5 g/l glucose, 20% FBS, 10% DMSO

Required Selective Antibiotic(s)
- 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.
3. Transfer cells in 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% CO2.

Frozen Stock Preparation
1. Resuspend cells at a density of 3-5 x 10^6 cells/ml in freezing medium prepared extemporaneously with cold growth medium.
2. Aliquot 1 ml cells 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.

Cell Maintenance
1. Maintain and subculture the cells in growth medium supplemented with 200 µg/ml Zeocin™.
2. Renew growth medium twice a week.
3. Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

Cell-Handling Recommendations
To ensure the best results:
- Use RAW-Lucia™ ISG-KO-cGAS cells with less than 20 passages.
- Pass cells either 3 or 4 days prior to the reporter assay.
- Three days prior to the reporter assay, seed cells at a cell density of 2.5x10^4 per cm², which corresponds to 1.9x10^6 cells in a T-75 culture flask.
- Four days prior to the reporter assay, seed cells at a cell density of 1.3x10^6 per cm², which corresponds to 1x10^6 cells in a T-75 culture flask.

Reporter Assay
Day 1:
1. Sample preparation: Prepare a working dilution range of your samples in endotoxin-free water.
2. Cell suspension preparation:
   - Remove medium from RAW-Lucia™ ISG-KO-cGAS cells and rinse twice with warm PBS.
   - Use a cell scraper to detach cells and resuspend cells in growth medium and prepare a cell suspension at ~550,000 cells/ml.
3. Add 20 µl of your sample in a well of a flat-bottom 96-well plate.
4. Add 20 µl of a positive control (such as murine Type I IFN) in another well.
5. Add 20 µl of negative control (or growth medium) in another well.
6. Add 180 µl of cell suspension (~100,000 cells) per well.

Day 2:
1. Prepare QUANTI-Luc™ following the instructions on the pouch.
2. 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.
3. Pipet 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.
4. Prime the injector with the QUANTI-Luc™ assay solution and proceed with the measurement.

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