RAW-Lucia™ ISG-KO-MAVS Cells

MAVS knockout IRF-inducible Lucia luciferase reporter mouse macrophages

Catalog # rawl-komavs

For research use only

Version # 16F01-MM

PRODUCT INFORMATION

Contents and Storage
• 1 vial of RAW-Lucia™ ISG-KO-MAVS 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-MAVS cells should not be passaged more than 20 times to remain fully efficient. RAW-Lucia™ ISG-KO-MAVS 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
• MAVS knockout has been verified by functional assays (see validation sheet) 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.

INTRODUCTION

Mitochondrial antiviral-signaling protein (MAVS; also known as IPS-1, CARDIF, VISA) is an adaptor protein that plays a critical role in the immune response to viral infection. The innate immune system senses intracellular double-stranded RNA (dsRNA), a replication intermediate for RNA viruses, through two RNA helicases: retinoic acid inducible gene-I (RIG-I) and melanoma differentiation-association gene 5 (MDA5). These two sensors recognize different ligands, yet both signal through MAVS. Specifically, upon recognition of dsRNA, they are recruited by MAVS to the outer membrane of the mitochondria leading to the activation of interferon regulatory factor 3 (IRF3), which in turn regulates the expression of type I interferons (IFNs).

RAW-Lucia™ ISG-KO-MAVS and RAW-Lucia™ ISG cells can be used to study the role of MAVS by monitoring of IRF-induced Lucia luciferase activity. They express the gene for secreted Lucia luciferase under the control of an IFN-inducible IFN-stimulated genes 54 (ISG54) promoter enhanced by a multimeric IFN-stimulated response elements (ISRE). The levels of IRF-induced Lucia in the cell culture supernatant can be easily monitored using QUANTI-Luc™, a Lucia luciferase detection reagent.

RAW-Lucia™ ISG-KO-MAVS cells were generated from RAW-Lucia™ ISG cells through the stable knockout of the MAVS gene. These cells derive from the murine RAW 264.7 macrophage cell line, which has been reported to express many pattern recognition receptors (PRRs), including the dsRNA sensors MDA-5 and RIG-I along with their adaptor protein MAVS.

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


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, 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.

Note: All steps from this point should be carried out under strict aseptic conditions.
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 10⁶ cells/ml in freezing medium prepared extemporaneously with cold growth medium.

Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.
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.

Note: If properly stored, cells should remain stable for years.

Cell Maintenance
1. Maintain and subculture the cells in growth medium supplemented with 200 µg/ml Zeocin™.

Note: We recommend the use of Pen-Strep (50 U/ml-50 µg/ml) together with Normocin™ or Primocin™ to keep the cells free of microbial contaminants.
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.

Note: We recommend the use of a cell scraper to detach cells.

Cell-Handling Recommendations
To ensure the best results:
- Use RAW-Lucia™ ISG-KO-MAVS 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 2.5 x 10⁴ per cm², which corresponds to 1.9 x 10⁶ cells in a T-75 culture flask.
- Four days prior to the reporter assay, seed cells at a cell density of 1.3 x 10⁴ per cm², which corresponds to 1 x 10⁶ cells in a T-75 culture flask.

Reporter Assay
Day 1:
Use RAW-Lucia™ ISG-KO-MAVS cells with their corresponding parental (wild-type) cell line, RAW-Lucia™ ISG cells.

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-MAVS 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 IFN-α (1 x 10⁴ IU/ml final concentration) in another well.
5. Add 20 µl of a RIG-I ligand such as 5’ppp-dsRNA/LyoVec™ (300 ng/ml final concentration) in another well.

Note: This ligand will induce Lucia luciferase activity in the parental RAW-Lucia™ ISG cells but not in RAW-Lucia™ ISG-KO-MAVS cells.
6. Add 20 µl of negative control such as growth medium in another well.
7. Add 180 µl of cell suspension (~100,000 cells) per well.

IMPORTANT: To ensure reliable and reproducible results, homogenize the cell suspension.
8. Incubate the plate at 37°C in a 5% CO2 incubator for 18-24 h.

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-MAVS cell culture supernatant per well in a 96-well flat-bottom (opaque) or black plate, or a luminometer tube.
4. Prime the injector with the QUANTI-Luc™ assay solution and proceed with the measurement.

RELATED PRODUCTS

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<thead>
<tr>
<th>Product</th>
<th>Catalog Code</th>
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<tr>
<td>RAW-Lucia™ ISG (Parental cell line)</td>
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