RAW-ASC KO-NLRC4 Cells

NLRC4 knockout murine macrophages

Catalog code: raw-konlrc4

https://www.invivogen.com/raw-konlrc4

For research use only

Version 21A04-NJ

PRODUCT INFORMATION

Contents

- 3-7 x 10^6 RAW-ASC KO-NLRC4 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 Blasticidin** (10 mg/ml). Store at 4°C or -20°C.*
- **1 ml of Normocin™** (50 mg/ml)*. Normocin™ is a formulation of three antibiotics to prevent contamination from mycoplasmas, 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.

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

Genetic instability is a biological phenomenon that occurs in all stably transfected cells, resulting in reduced responsiveness in normal cell culture conditions. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages.

Quality Control

- Expression of murine *ASC* has been verified by PCR and Western blot (WES™).
- Biallelic murine *NLRC4* knockout has been verified by DNA sequencing, PCR, Western blot (WES™), and functional assays.
- The stability for 20 passages, following thawing, has been verified.
- These cells are guaranteed mycoplasma-free.

USE RESTRICTIONS

These cells are distributed for research purposes only.

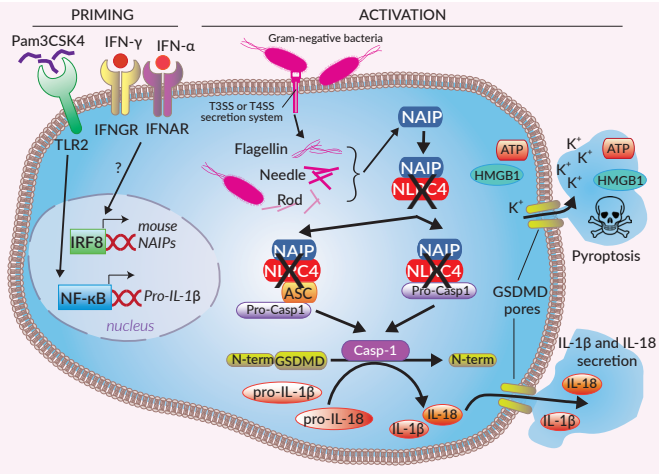
This product is covered by a Limited Use License. For non-research use, such as screening, quality control or clinical development, please contact info@invivogen.com.

PRODUCT DESCRIPTION

RAW-ASC KO-NLRC4 cells were generated from the RAW-ASC cell line, which derives from the naturally ASC deficient RAW 264.7 macrophage cell line[1]. RAW-ASC KO-NLRC4 cells stably express the transfected murine *ASC* gene and have a stable knockout of the *NLRC4* gene. These cells are fully KO for the NLRC4 protein. Mature IL-1β secretion and pyroptosis are abolished in these cells after activation of the NLRC4 inflammasome (e.g. LFn-Rod). These cells are resistant to Blasticidin.

BACKGROUND

Inflammasomes are cytoplasmic multi-protein complexes, characterized by a primary sensor, that assemble in response to infections and cellular damage. NLRC4 (Nucleotide-binding domain (NBD) and leucin-rich repeat (LRR) receptor, CARD domain-containing protein 4, or IPAF) senses intracellular bacterial molecules such as Flagellin from the motility apparatus, or Inner Rod, and Needle proteins from the type III or IV secretion systems (T3SS or T4SS). More specifically, NLRC4 associates with NAIP (NLR family apoptosis inhibitory protein) which directly binds to the ligands. Mice express multiple NAIPs which display different affinity for each ligand. NAIP1 exhibits a higher affinity for T3SS Needle, NAIP2 for T3SS Inner Rod, and NAIP5 and NAIP6 for Flagellin[2-3]. Although the transcriptional regulation of NAIPs and NLRC4 is poorly documented, the basal expression of murine NAIPs has recently been found to depend at least on IRF8[3]. Once recruited by NAIP, NLRC4 triggers homo-polymerization, resulting in the clustering of NLRC4 CARD domains. The NLRC4/NAIP complex then associates with pro-caspase-1, either via direct CARD-CARD interactions, and/or through the ASC (apoptosis-associated speck-like protein) adaptor[1]. Activation of caspase-1 induces the cleavage of pro-IL-1β/pro-IL-18, formation of gasdermin D (GSDMD) pores, secretion of IL-1β/-18, and pyroptosis[4,5]. The NLRC4 inflammasome appears to protect mucosal barriers such as the lung, stomach, and intestine from invading bacteria[5]. Gain-of-function mutations have been described in human NLRC4 and are associated with auto-inflammatory conditions[5].



1. Pelerin P. *et al.*, 2008. P2X7 receptor differentially couples to distinct release pathways for IL-1β in mouse macrophage. J. Immunol. 180:7147. 2. Tenthorey, J.L. *et al.*, 2014. Molecular Basis for Specific Recognition of Bacterial Ligands by NAIP/NLRC4 Inflammasomes. Mol Cell. 54:17-29. 3. Karki, R., *et al.*, 2018. IRF8 Regulates Transcription of Naips for NLRC4 Inflammasome Activation. Cell, 173(4):920-933. e13 4. Zhang L. *et al.*, 2015. Cryo-EM structure of the activated NAIP2-NLRC4 inflammasome reveals nucleated polymerization. Science. 350:404-409. 5. Bauer R. & Rauch I., 2020. The NAIP/NLRC4 inflammasome in infection and pathology. Mol. Aspects Med. Jun 1:100863.

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

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HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** DMEM, 4.5 g/l glucose, 4 mM L-glutamine, 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml Normocin™

Note: The use of Normocin™ together with Pen-Strep is required to keep the cells free of microbial contaminants. Contamination of this cell line may activate PRRs, such as TLRs, resulting activation of PRR signaling pathways.

- **Test Medium:** DMEM **without phenol red**, 4.5 g/l glucose, 4 mM L-glutamine, 10% heat-inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, **without Normocin™ and Blasticidin**

Note: Phenol red causes high background signal in the LDH (lactate dehydrogenase) assay used to monitor inflammasome-induced cell death.

- **Freezing Medium:** DMEM, 20% FBS and 10% DMSO
- **Required Selective Antibiotic:** Blasticidin

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: From this point strict aseptic conditions must be used.

3. Transfer cells to a larger tube containing 15 ml of pre-warmed growth medium. **Do not add selective antibiotics until the cells have been passaged twice.**
4. Centrifuge at RCF 150 g for 10 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium without selective antibiotic.
6. Transfer cells to a T-25 culture flask containing 5 ml of growth medium and place the culture at 37 °C in 5% CO_2.

Cell Maintenance

1. RAW-ASC KO-NLRC4 cells are adherent cells. Gently, use a cell scraper to detach the cells. Do **not** use trypsin.
2. After cells have recovered, subculture the cells in growth medium with an initial seeding density of 1.5 x 10^4 cells/cm^2 (e.g. ~ 1 x 10^6 cells in a T-75 culture flask). To maintain selection pressure, add 10 µg/ml of Blasticidin to the growth medium.
3. Renew growth medium twice a week.
4. Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

Note: The average doubling time for the RAW-ASC KO-NLRC4 cells is ~29 hours using the conditions described above.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10^6 cells/ml in freshly prepared freezing medium with cold DMEM.

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

2. Dispense 1 ml of the 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-ASC KO-NLRC4 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.

SAFETY CONSIDERATIONS

Biosafety Level 2

EXPERIMENTAL PROCEDURES

RAW-ASC KO-NLRC4 cells are designed to study the signals involved in inflammasome activation. Below is an example protocol to induce canonical and non-canonical inflammasome responses using the RAW-ASC KO-NLRC4 cell line and the parental RAW-ASC cell line.

Cell preparation

1. Five days prior the assay, seed cells at 5 x 10^3 cells/cm^2 (corresponding to 2.5 x 10^4 cells in 15ml in a T-75 culture flask) in growth medium.
2. On the day of the assay, gently detach cells using a cell scraper.
3. Centrifuge at RCF 300g for 5 minutes.
4. Remove supernatant and resuspend cells in freshly prepared, pre-warmed **test medium** at 1.1 x 10^6 cells/ml.

Pre-priming (for non-canonical inflammasome induction only)

1. Dispense 20 µl of recombinant murine (m)IFN-γ at 100 ng/ml (final concentration: 10 ng/ml) per well of a flat-bottom 96-well plate.
2. Add 180 µl of cell suspension (~200,000 cells) per well.
3. Incubate the plate for 18-24h at 37 °C in 5% CO_2.

Priming

1. Dispense 20 µl of Pam3CSK4 at 1 µg/ml (final conc: 100 ng/ml) per well of a flat-bottom 96-well plate.

Note: For non-canonical inflammasome induction, add Pam3CSK4 directly to pre-primed cells (20 µl at 1 µg/ml per well).

2. Add 180 µl of cell suspension (~200,000 cells) per well.
3. Incubate the plate for 3h at 37 °C in 5% CO_2.

Activation

1. Carefully remove culture supernatant. Add 180 µl of test medium.
2. Add 20 µl of inflammasome inducer per well.

Note: We recommend to perform a dilution series for each inducer (e.g. 1:5 dilution series of LFn-Rod starting at 10 µg/ml. LFn-Rod must be used in combination with the anthrax protective antigen. Please refer to the LFn-Rod technical data sheet.)

3. Include a negative control (no inducer).
4. Incubate the plate for 6h at 37°C in 5% CO_2.
5. Take 100 µl of culture supernatant for analysis of murine (m)IL-1β secretion and/or cell death.

Optional: These samples can be stored at -80°C until required.

6. Add 100 µl of test medium to each well of the original culture plate and continue to incubate for an additional 18h at 37°C in 5% CO_2.
7. Take 100 µl of culture supernatant for analysis of mIL-1β secretion and/or cell death.

Optional: These samples can be stored at -80°C until required.

Detection of mature mIL-1β and cell death in supernatant

- Inflammasome-induced secretion of bioactive mIL-1β in the RAW-ASC KO-NLRC4 cell culture supernatant can be monitored using a murine IL-1β ELISA kit.
- Cell death can be monitered using classical assays such as the lactate dehydrogenase (LDH) assay, following the manufacturer's instructions.

RELATED PRODUCTS

Product	Description	Cat. Code
Blasticidin	Selective antibiotic	ant-bl-05
LFn-Rod	Inflammasome inducer	tlrl-rod
Poly(dA:dT)	Inflammasome inducer	tlrl-patn
Pam3CSK4	TLR1/2 agonist	tlrl-pms
RAW-ASC cells	Inflammasome test cells	raw-asc

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