RAW-ASC KO-GSDMD Cells

Gasdermin D knockout murine macrophages

Catalog code: raw-kogsdmd

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

For research use only

Version 20G30-NJ

PRODUCT INFORMATION

Contents

- 3-7 x 10^6 RAW-ASC KO-GSDMD 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 *GSDMD* knockout has been verified by PCR, Western blot (WES™), and functional assays.
- The stability for 20 passages, following thawing, has been verified.
- These cells are guaranteed mycoplasma-free.

SAFETY CONSIDERATIONS

Biosafety Level 2

PRODUCT DESCRIPTION

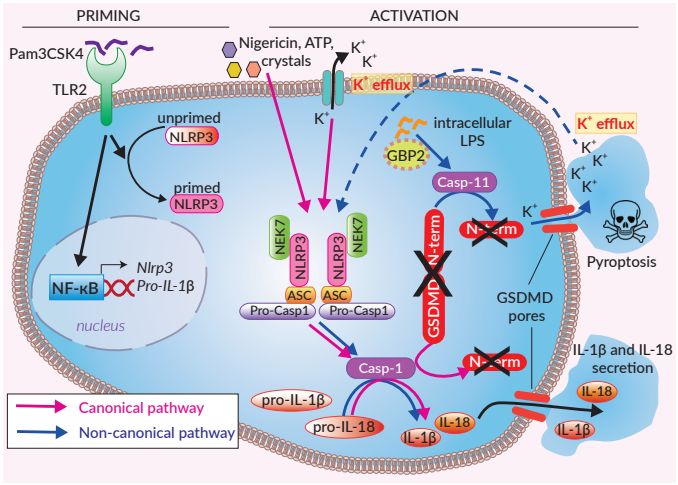
RAW-ASC KO-GSDMD cells were generated from the RAW-ASC cell line, which derives from the naturally ASC deficient RAW 264.7 macrophage cell line[1]. RAW-ASC KO-GSDMD cells stably express the transfected murine *ASC* gene and have a stable knockout of the gasdermin D (*GSDMD*) gene. Mature IL-1β secretion and pyroptosis is abolished in RAW-ASC KO-GSDMD cells, upon activation of canonical (NLRP3 and AIM2) and non-canonical (caspase-11) inflammasomes. Of note, longer inflammasome activation (24 hours) can induce some cell death, most likely through secondary necrosis of apoptotic cells[2].

These cells are resistant to blasticidin.

BACKGROUND

Gasdermin D (GSDMD) is a cytoplasmic pore-forming protein that has been described as a major actor in inflammasome responses[3]. GSDMD belongs to the gasdermin family, found in both humans and mice, and is expressed in immune and intestinal epithelial cells[3,4]. GSDMD consists of two distinct domains, whereby the C-terminal domain exerts an auto-inhibitory function on the N-terminal domain. Proteolytic cleavage of GSDMD by activated caspase-1 (CASP1) or murine CASP11 (CASP4/5 in humans) allows the release of the N-terminal domain, which oligomerizes to form 10-15 nm diameter pores at the cell membrane. These pores allow the release of alarmins (e.g. HMGB1) and the secretion of mature IL-1β and IL-18 inflammatory cytokines. The accumulation of GSDMD pores in the membrane causes cell swelling and rupture, leading to an inflammatory cell death termed pyroptosis[3,4].

Importantly, upon intracellular lipopolysaccharide (LPS) recognition by CASP11-4/5, GSDMD pores form and lead to stress signals such as cytosolic ion concentration imbalances (i.e. K+ efflux). This activates the NLRP3 sensor and the canonical inflammasome. Thereby, GSDMD links the non-canonical and canonical inflammasome responses[5,6].



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. Kayagaki N. et al., 2015.** Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. Nature. 526:666. **3. Feng S. *et al.*, 2018.** Mechanisms of Gasdermin family members in inflammasome signaling and cell death. J. Mol. Biol. 430:3068. **4. Kovacs S.B. & Miao E.A. 2017.** Gasdermins: effectors of pyroptosis. Trends Cell. Biol. 27:673. **5. Groslambert M. & Py B. 2018.** Spotlight on the NLRP3 inflammasome pathway. J. Inflamm. Res. 11:359. **6. Mathur A. *et al.*, 2017.** Molecular mechanisms of inflammasome signaling. J. Leuk. Biol. 103:233.

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.

TECHNICAL SUPPORT

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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 in 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 deshydrogenase) 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 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 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-GSDMD 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-GSDMD cells is ~31 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-GSDMD 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.

EXPERIMENTAL PROCEDURES

RAW-ASC KO-GSDMD 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 RAW-ASC KO-GSDMD cells 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 1000-1200 RPM (RCF 200-300 g) 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. 1:2 dilution series of Nigericin starting at 10 µM).

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-GSDMD 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
E. coli OMVs	Inflammasome inducer	tlrl-omv-1
Nigericin	Inflammasome inducer	tlrl-nig
Poly(dA:dT)	Inflammasome inducer	tlrl-patn
LPS-EB (*E. coli* 0111:B4)	TLR4 agonist	tlrl-3pelps
Pam3CSK4	TLR1/2 agonist	tlrl-pms
RAW-ASC cells	Inflammasome test cells	raw-asc
RAW-ASC KO-CASP11 cells	Inflammasome test cells	raw-kocasp11

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