A549-ASC Cells

ASC-GFP reporter lung carcinoma cells

Catalog code: a549-ascg

https://www.invivogen.com/a549-ascg-nlrp1

For research use only

Version 22I21-AK

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10^6 **A549-ASC 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 at -20 °C.*
- **1 ml of Normocin™** (50 mg/ml), a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20 °C.*

*The expiry date is specified on the product label.

Handling 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

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 genetically engineered cells. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages. To ensure maximum efficiency, do not passage A549-ASC cells more than 20 times.

Quality Control

- The induction of ASC::GFP expression has been verified by Western blot, fluorescence microscopy and flow cytometry.
- The stability of this cell line for 20 passages following thawing has been verified.
- A549-ASC 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 to 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.

PRODUCT DESCRIPTION

A549-ASC cells are designed as a control cell line for A549-ASC-NLRP1 cells to study the activation of the NLRP1 inflammasome in real time using fluorescence microscopy. They were engineered from the human A549 lung carcinoma epithelial cell line to express an ASC::GFP fusion protein under the control of an NF-κB-inducible promoter.

A549 cells endogenously express proteins involved in the inflammasome signaling, including ASC, caspase-1, and Gasdermin D/E. However, they are unable to mount inflammasome responses because of the lack of expression of some sensors/co-sensors (in-house data)[1]. A549-ASC cells may be used to monitor ASC-dependent inflammasome activation after transgenic expression of a desired inflammasome sensor/co-sensor.

Besides, InvivoGen provides A549-ASC-NLRP1 cells to specifically assess NLRP1 inflammasome activation. The formation of NLRP1 inflammasome is strictly dependent on the ASC adaptor to bridge the sensor interaction with pro-caspase-1[2]. Upon NLRP1 activation, the ASC::GFP speck formation can be visualized using fluorescence microscopy. Of note, the green fluorescent protein (GFP) is fused to the C-terminal region of the ASC protein. It does not prevent the formation of inflammasomes and downstream responses.

A549-ASC cells are resistant to Blasticidin.

BACKGROUND

ASC (apoptosis-associated speck-like protein containing a CARD domain, aka PYCARD) is a protein adaptor important in canonical inflammasome responses. ASC's bipartite composition, consisting of one PYD (pyrin domain) and one CARD (caspase recruitment domain) domain, allows the recruitment of the CARD-containing pro-caspase-1 to canonical inflammasome sensors[3]. In resting cells, ASC is present in a soluble and diffuse form, both in the cytoplasm and nucleus. Upon inflammasome activation, ASC molecules form a single large, micrometer-sized, 'speck' per cell, thus concentrating caspase-1 activation sites[4].

1. Planès *et al.*, 2022. Human NLRP1 is a sensor of pathogenic coronavirus 3CL proteases in lung epithelial cells. Mol Cell. S1097-2765(22). **2. Taabazuing C.Y. *et al.*, 2020.** The NLRP1 and CARD8 inflammasomes. Immunol. Reviews. 297(1):13-25. **3. Mathur A. *et al.*, 2017.** Molecular mechanisms of inflammasome signaling. J. Leuk. Biol. 103:233. **4. Hoss F. *et al.*, 2017**. Assembly and regulation of ASC specks. Cell. Mol. Life Sci. 74:1211.

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SAFETY CONSIDERATIONS

Biosafety Level 1

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 25 mM HEPES, 10% (v/v) heat-inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml Normocin™.
- **Freezing Medium:** DMEM, 20% FBS, 10% DMSO.
- **Test Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 25 mM HEPES, 10% (v/v) heat-inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, **without antibiotics.**

Note: Heat-inactivated FBS is also commercially available.

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 must 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 vial containing 15 ml of pre-warmed growth medium. **Do not add selection antibiotics until the cells have been passaged twice.**
4. Centrifuge vial at 150 x g (RCF) for 10 min.
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.

Note: To avoid excessive alkalinity of the medium during recovery of the cells, place the tissue culture flask containing the growth medium into an incubator for at least 15 minutes before adding the vial contents.

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 freshly prepared freezing medium with cold growth medium.

Note: A T-75 culture flask typically yields enough cells for preparing 1-2 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. A549-ASC cells grow as adherent cells. To detach cells, rinse the cell layer with PBS, then incubate with trypsin-EDTA for 2-5 min. Do not use a cell scraper.
2. Maintain and subculture the cells in growth medium supplemented with 10 µg/ml of Blasticidin.
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 A549-ASC cells is 24-36 hours using the conditions described above.

ACTIVATION OF A549-ASC CELLS

Below is a protocol using A549-ASC cells as a control cell line for A549-ASC-NLRP1 cell activation. *For more information, please visit https://www.invivogen.com/a549-ascg-nlrp1.* It is recommended to perform the assay with test medium which does not contain Normocin™, nor Blasticidin.

Day 1: Cell preparation

1. Prepare a suspension of A549-ASC-NLRP1 cells, and A549-ASC control cells in test medium at 2.5 x 10^5 cells/ml.
2. Add 200 µl of the cell suspension per well of a flat-bottom 96-well plate (~5.0 x 10^4 cells/well).
3. Incubate overnight at 37°C in 5% CO_2.

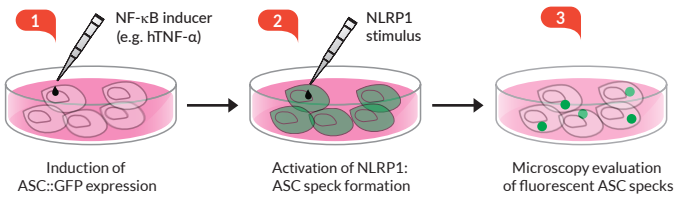
Day 2: Induction of ASC::GFP expression

1. Carefully remove cell supernatant.
2. Add 180 µl of freshly made test medium.
3. Add 20 µl of NF-κB-inducer, such as hTNF-α (final conc. 4 ng/ml), per well.
4. Incubate overnight at 37°C in 5% CO_2.

Day 3: Induction and visualization of ASC speck formation

1. Carefully remove cell supernatant.
2. Add 180 µl of freshly made test medium.
3. Add 20 µl of NLRP1 activator, such as Val-boroPro (final conc. 10 µM), per well.
4. Incubate for 8 hours at 37°C in 5% CO_2.
5. Monitor fluorescent ASC signal in real-time using fluorescence microscopy and normal FITC filter sets.

Note: Fluorescent speck formation requires a transgenic expression of an inflammasome sensor (e.g. NLRP1). The incubation time required to visualize ASC specks depends on the type of inflammasome sensor, and the type and concentration of inflammasome activator.



Spectral properties of GFP

Excitation λ max:	480 nm
Emission λ max:	505 nm

RELATED PRODUCTS

Product	Cat.code
A549-ASC-NLRP1 cells	a549-ascg-nlrp1
Human TNF-α	rcyc-htnfa
Val-boroPro	tlrl-vbp-10
Poly(I:C) HMW	tlrl-pic
Blasticidin	ant-bl-05
Normocin™	ant-nr-1

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