

A549-ASCoV2-NLRP1 Cells

ASC-GFP reporter lung carcinoma cells expressing the human ACE2, TMPRSS2, and NLRP1 genes

Catalog code: a549-ascov2-nlrp1

<https://www.invivogen.com/a549-ascg-cov>

For research use only

Version 22H04-NJ

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10⁶ A549-ASCoV2-NLRP1 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-ASCoV2-NLRP1 cells more than 20 times.

Quality Control

- The induction of ASC::GFP expression has been verified by western blot and fluorescence microscopy.
- The overexpression of the human ACE2 (*hACE2*) gene has been verified by flow cytometry and functional assays.
- The overexpression of the human TMPRSS2 (*hTMPRSS2*) gene has been verified by RT-qPCR and functional assays.
- The overexpression of the human NLRP1 (*hNLRP1*) gene has been verified by western blot and functional assays.
- The stability of this cell line for 20 passages following thawing has been verified.
- A549-ASCoV2-NLRP1 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.

TECHNICAL SUPPORT

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

PRODUCT DESCRIPTION

A549-ASCoV2-NLRP1 cells are designed to study the activation of the NLRP1 inflammasome in real time, in the context of SARS-CoV-2 infection.

A549-ASCoV2-NLRP1 cells were engineered from the human A549 lung carcinoma epithelial cell line to stably express the SARS-CoV-2 receptors, ACE2 and TMPRSS2. They are thus highly permissive to infection.

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-ASCoV2-NLRP1 cells feature stable expression of the human NLRP1 (isoform 1) gene. The formation of NLRP1 inflammasome is strictly dependent on the ASC adaptor to bridge the sensor interaction with pro-caspase-1¹. A549-ASCoV2-NLRP1 cells additionally express an ASC::GFP fusion protein under the control of an NF-κB-inducible promoter. Thus, these cells allow the visualization of NLRP1 inflammasome activation by monitoring GFP emission and subsequent ASC speck formation using fluorescence microscopy.

As a control cell line, InvivoGen offers [A549-ASCoV2 cells](#).

A549-ASCoV2-NLRP1 cells are resistant to [Blasticidin](#).

BACKGROUND

NLRP1 (NOD-like receptor pyrin domain-containing protein 1) was the first described inflammasome sensor of the NLR family in 2001². Its cognate ligands have only recently been discovered, including long dsRNA sensing, viral protease-dependent cleavage of NLRP1 or pharmacological inhibition of the NLRP1 regulators DPP8/9¹.

ASC (apoptosis-associated speck-like protein containing a CARD domain, aka PYCARD) is a protein adaptor important in canonical inflammasome responses^{3,4}. 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 cell⁴.

ACE2 (angiotensin I-converting enzyme-2) and TMPRSS2 (transmembrane protease serine 2) are cell surface proteins that both interact with the SARS-CoV-2 Spike (S) protein, allowing the virus entry into target cells⁵.

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. Martinon *et al.* 2002. A molecular platform triggering activation of inflammatory caspases and processing of proIL-β. *Mol Cell*. 10(2):417. 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. 5. Hoffmann M. *et al.*, 2020. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*. 181:1.



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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% CO₂.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10⁶ 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-ASCoV2-NLRP1 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 A549-ASCoV2-NLRP1 cells is 24 hours using the conditions described above.

TECHNICAL SUPPORT

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ACTIVATION OF A549-ASCoV2-NLRP1 CELLS

Below is a protocol using **A549-ASCoV2-NLRP1** cells for monitoring NLRP1 inflammasome formation upon SARS-Cov-2 infection. For more information, please visit <https://www.invivogen.com/a549-ascg-cov>. It is recommended to perform the assay with test medium which does not contain **Normocin™**, nor **Blasticidin**.

Day 1: Cell preparation

1. Prepare a cell suspension of **A549-ASCoV2-NLRP1** cells and **A549-ASCoV2** control cells in test medium at 2.5 x 10⁵ cells/ml.
2. Add 200 µl of the cell suspension per well of a flat-bottom 96-well plate (~ 5 x 10⁴ cells/well).
3. Incubate overnight at 37°C in 5% CO₂.

Day 2: Induction of ASC::GFP expression (Optional)

Note: It has been observed that A549-ASCoV2-NLRP1 cells express low levels of cytoplasmic ASC::GFP, which is increased upon cell priming using hTNF-α (see data on our website).

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. 0.5 ng/ml), per well.

Note: Depending on the application, hTNF-α concentration for priming should be optimized by the user.

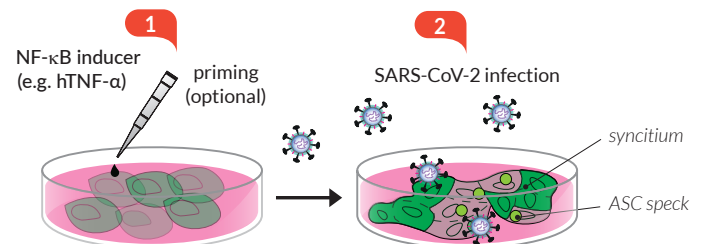
4. Incubate overnight at 37°C in 5% CO₂.

Day 3: Cell infection with SARS-CoV-2

1. Carefully remove cell supernatant.
2. Add 50 µl of SARS-CoV-2 at optimal MOI (ranging from 0.01 to 1).
3. Incubate 1 hour at 37°C in 5% CO₂.
4. Carefully remove cell supernatant.
5. Add 200 µl of fresh test medium
6. Incubate overnight at 37°C in 5% CO₂.

Day 4: Visualization of ASC::GFP expression

Monitor fluorescent ASC signal in real-time using fluorescence microscopy and normal FITC filter sets.



Activation of A549-ASCoV2-NLRP1 cells

Spectral properties of GFP:

Excitation λ max: 480 nm

Emission λ max: 505 nm

RELATED PRODUCTS

Product	Cat.code
A549-ASCoV2 cells	a549-ascg-cov
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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