A549-ASCoV2 Cells

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

Catalog code: a549-ascov2

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

For research use only

Version 22H04-NJ

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10^6 **A549-ASCoV2 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 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 stability of this cell line for 20 passages following thawing has been verified.
- A549-ASCoV2 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-ASCoV2 cells are designed as a control cell line for A549-ASCoV2-NLRP1 cells to study the activation of the NLRP1 inflammasome in real time, in the context of SARS-CoV-2 infection.

They 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. Moreover, they express an ASC::GFP fusion protein under the control of an NF-κB-inducible promoter. This allows the monitoring of ASC speck formation using fluorescence microscopy.

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 cells not only serve as a control cell line for A549-ASCoV2-NLRP1 cells but also offer the possibility to monitor ASC-dependent inflammasome activation after transgenic expression of desired sensor/co-sensor. 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-ASCoV2 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[2]. It consists of one PYD (pyrin domain) and one CARD (caspase recruitment domain) domain, which allows the recruitment of the CARD-containing pro-caspase-1 to canonical inflammasome sensors[2]. 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[3].

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 to enter into target cells[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. Mathur A. *et al.*, 2017.** Molecular mechanisms of inflammasome signaling. J. Leuk. Biol. 103:233. **3. Hoss F. *et al.*, 2017.** Assembly and regulation of ASC specks. Cell. Mol. Life Sci. 74:1211. **4. 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 CONSIWDERATIONS

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-ASCoV2 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 cells is 24 hours using the conditions described above.

ACTIVATION OF A549-ASCoV2 CELLS

Below is a protocol using A549-ASCoV2 cells as a control cell line for A549-ASCoV2-NLRP1 cell activation by SARS-Cov-2. *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 10^5 cells/ml.
2. Add 200 µl of the cell suspension per well of a flat-bottom 96-well plate (~ 5 x 10^4 cells/well).
3. Incubate overnight at 37°C in 5% CO_2.

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

Note: It has been observed that A549-ASCoV2 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.

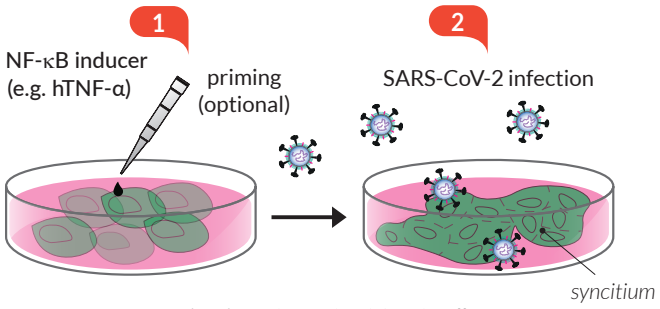
4. Incubate overnight at 37°C in 5% CO_2.

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% CO_2.
4. Carefully remove cell supernatant.
5. Add 200 µl of fresh test medium.
6. Incubate overnight at 37°C in 5% CO_2.

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 cells

Spectral properties of GFP:
Excitation λ max: 480 nm
Emission λ max: 505 nm

RELATED PRODUCTS

Product	Cat.code
A549-ASCoV2-NLRP1 cells	a549-ascg-cov-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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