A549-hACE2 Cells

A549 lung carcinoma cells expressing the human ACE2 gene

Catalog code: a549-hace2

https://www.invivogen.com/a549-hace2tmprss2-cells

For research use only

Version 20K23-NJ

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10^6 A549-hACE2 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 Puromycin** (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.

Note: Data sheets for all components are available on our website.

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

Quality Control

- The overexpression of the human *ACE2* (hACE2) gene has been verified by RT-qPCR, FACS staining, and functional assays.
- The stability for 20 passages following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

SAFETY CONSIDERATIONS

Biosafety Level 1

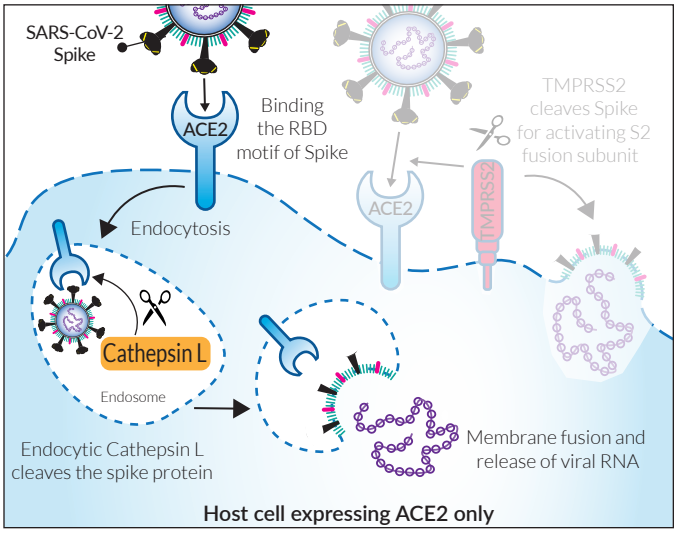
CELL LINE DESCRIPTION

A549-hACE2 cells were generated from the A549 lung carcinoma cell line, a commonly used cellular model for the study of respiratory infections. A549-hACE2 cells were stably transfected to express the human *ACE2* (hACE2) gene. Thus, unlike their parental cell line, they are permissive to infection with pseudotyped lentiviruses expressing the SARS-CoV-2 Spike protein. A549-hACE2 cells are resistant to Puromycin.

The additional expression of the human TMPRSS2 gene in the A549-hACE2-TMPRSS2 cells significantly increases their permissivity to infection by SARS-CoV-2 Spike-pseudotyped lentiviruses.

BACKGROUND

ACE2 (angiotensin I-converting enzyme-2) is a type I membrane protein that belongs to the angiotensin-converting enzyme family[1]. It is expressed in arteries, heart, kidneys, and epithelia of the lung and small intestine[2]. Human ACE2 is the established host receptor for the Spike (S) protein of SARS-CoV-2, the causative agent of COVID-19, enabling its entry into target cells[3-5]. In particular, SARS-CoV-2 gains entry to host cells through the binding of the Spike receptor-binding domain (RBD) to ACE2 at the cell surface[4,5]. Following this, host proteases, such as TMPRSS2 and Cathepsin L, allow the cleavage of the S protein into two subunits (S1 and S2), at the cell surface or in the endosomes, respectively. S2 mediates the fusion between the viral and host membranes, thereby releasing the viral contents into the cell.[4-6].



1. Donoghue M. *et al.*, 2000. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Cir. Research. 87(5):e1-e9. **2. Harmer D. *et al.*, 2002.** Quantitative mRNA expression profiling of ACE2, a novel homolog of angiotensin-converting enzyme. FEBS Letters. 532(1-2):107-110. **3. Li W. *et al.*, 2003.** Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 426(6965):450-454. **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. 181:1-16. **5. Zhou P. *et al.*, 2020.** A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 579(7798):270-273. **6. Walls A.C. *et al.*, 2020.** Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell. 181(2):281-292.e6.

APPLICATIONS

A549-hACE2 cells are permissive to infection by SARS-CoV-2 and/or spike-pseudotyped lentiviral particles. Thus, they are ideal for studying viral entry into host cells, as well as for screening small molecule inhibitors and neutralizing antibodies. These cells can be used for comparative studies with A549-hACE2-TMPRSS2 cells which express both ACE2 and TMPRSS2 and are more permissive to SARS-CoV-2 infection than A549-hACE2.

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HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum (FBS; 30 min at 56 °C), 100 µg/ml Normocin™, Pen-Strep (100 U/ml-100 µg/ml)
- **Freezing Medium:** DMEM, 4.5 g/l glucose, 10% FBS, 10% DMSO
- **Required Selection Antibiotic:** Puromycin

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 (approximately 2 minutes).
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 of the steps from this point should be carried out under strict aseptic conditions.

3. Transfer cells to a larger tube containing 15 ml of pre-warmed growth medium. **Do not add selection antibiotics until the cells have been passaged twice.**
4. Centrifuge tube at 200-300 x *g* for 5 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium without selective antibiotics.
6. Transfer the contents to a T-25 tissue culture flask containing 5 ml of growth medium without selective antibiotics.
7. Place the culture at 37°C in 5% CO_2.

Frozen Stock Preparation

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

Note: A T-75 culture flask typically yields enough cells for preparing 1-2 frozen vials.

2. Dispense 1 ml of 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 maintenance

1. A549-hACE2 cells grow as adherent cells. To detach cells, rinse the cell layer with PBS, then incubate with 0.25% trypsin-EDTA for 2-5 minutes.
2. After cells have recovered and are growing well (following at least 2 passages), maintain and subculture the cells in growth medium supplemented with 0.5 µg/ml of Puromycin.
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-hACE2 cells is ~25 hours using the conditions described above.

Cell Handling Recommendations

To ensure the best results, use A549-hACE2 cells with less than 20 passages.

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.

RELATED PRODUCTS

Product	Cat. Code
Puromycin	ant-pr-1
A549-hACE2-TMPRSS2 cells	a549-hace2tpsa
pLV-SARS2-S-d19	plv-cov2-sd19
pLV-SARS2-S-d19 (D614G)	plv-cov2-sd19g
Spike-S1-Fc	fc-sars2-s1
Spike-S1-His	his-sars2-s1
Spike-RBD-Fc	fc-sars2-rbd
Spike-RBD-His	his-sars2-rbd