A549-hACE2-TMPRSS2 Cells

A549 lung carcinoma cells expressing the human ACE2 and TMPRSS2 genes

Catalog code: a549-hace2tpsa

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-TMPRSS2 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 Hygromycin B Gold** (100 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 overexpression of the human *TMPRSS2* (hTMPRSS2) gene has been verified by RT-qPCR and functional assays.
- The stability for 20 passages following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

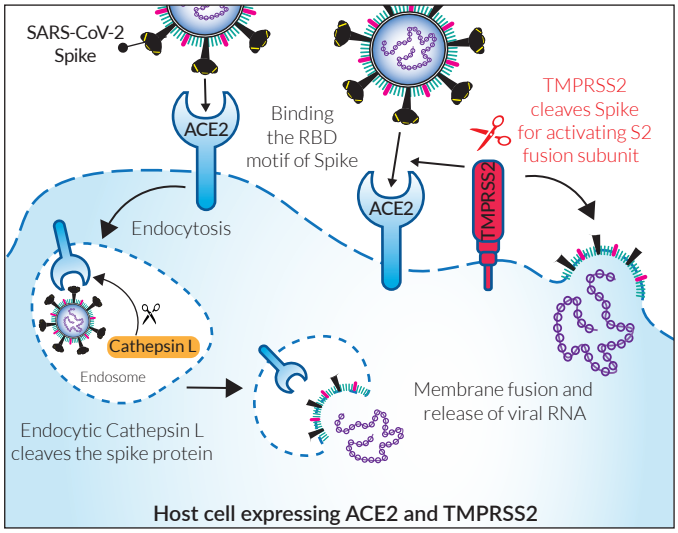
CELL LINE DESCRIPTION

A549-hACE2-TMPRSS2 cells were generated from the A549 lung carcinoma cell line, a commonly used cellular model for the study of respiratory infections. A549-hACE2-TMPRSS2 cells were stably transfected to express the human *ACE2* (hACE2) and *TMPRSS2* (hTMPRSS2) genes. These cells are more permissive to infection by pseudotyped lentiviruses expressing the SARS-CoV-2 Spike protein than A549-hACE2 cells, which only express hACE2.

A549-hACE2-TMPRSS2 cells are resistant to Puromycin and Hygromycin.

BACKGROUND

ACE2 (angiotensin I-converting enzyme-2) and TMPRSS2 (transmembrane protease serine 2) play a critical role in the pathogenesis of COVID-19 by allowing SARS-CoV-2 viral entry into target cells (e.g. human lung epithelium). ACE2 and TMPRSS2 are cell surface proteins that both interact with the virus Spike (S) protein[1-3]. ACE2 is mandatory for the binding of SARS-CoV-2 at the cell surface through its interaction with the Spike receptor-binding domain (RBD)[4]. Following this, TMPRSS2 cleaves the S protein into two functional subunits (S1 and S2), allowing virus-host membrane fusion, and the release of viral contents (e.g. RNA) into the cytosol[3-5]. Another protease, the Cathepsin L, also mediates cleavage of the S protein but it acts in the endosomes.



1. Chen H. *et al.*, 2020. SARS-CoV-2 activated lung epithelia cell proinflammatory signaling and leads to immune dysregulation in COVID-19 patients by single-cell sequencing. medRxiv: DOI 10.1101/2020.05.08.20096024. **2. 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. **3. Matsuyama S. *et al.*, 2020.** Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. PNAS. 117(13):7001-7003. **4. Zhou P. *et al.*, 2020.** A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 579(7798):270-273. **5. 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-TMPRSS2 cells are ideal for studying SARS-CoV-2 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 cells, which only express hACE2 and are less permissive to infection using SARS-CoV-2 Spike-pseudotyped viral particles.

TECHNICAL SUPPORT
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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, 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:** IMDM, 10% FBS, 10% DMSO
- **Required Selection Antibiotics:** Puromycin and Hygromycin

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-TMPRSS2 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 and 300 µg/ml of Hygromycin.
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-TMPRSS2 cells is ~25 hours using the conditions described above.

Cell Handling Recommendations

To ensure the best results, use A549-hACE2-TMPRSS2 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
Hygromycin B Gold	ant-hg-1
A549-hACE2 cells	a549-hace2
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

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