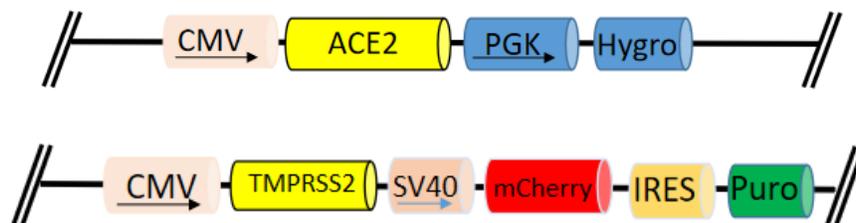


Datasheet for HEK293T/ACE2-TMPRSS2 Cell Line

- Catalog number:** SL222
- Product:** HEK293T cell line stably expressing angiotensin-converting enzyme 2 (ACE2) gene and transmembrane serine protease 2 (TMPRSS2) gene.
- Description:** The primary receptor for the human severe acute respiratory syndrome CoV-2 (SARS-CoV-2) virus is angiotensin-converting enzyme 2 (ACE2). Transmembrane protease/serine subfamily member 2 (TMPRSS2), a known human airway and alveolar protease, is required for efficient SARS-CoV-2 Spike (S) protein processing during entry into receptor cells. ACE2 and TMPRSS2 colocalize on cell surfaces and enhance the intracellular uptake of both SARS-CoV-2 virus and SARS-CoV-2 S-pseudotyped viruses (“pseudoviruses”).
- Quantity:** 1 vial of 2×10^6 cells; frozen
- Shipping conditions:** Dry ice
- Storage conditions:** Liquid nitrogen vapor phase. Remove the item from the dry ice packaging and check all items for damage and leakage. Place immediately into storage at or below -140°C , preferably into the liquid nitrogen vapor phase, until use.

Transgene integration:



Source of parental line:

HEK293T
Organism: *Homo sapiens*,
Human tissue: kidney
Cell type: epithelial

Quality control: >95% viability before freezing. All cells were tested and found to be free of mycoplasma, bacteria, viruses, and other toxins.

Safety instructions: To ensure safety, protective gloves, clothing, and a face mask should be worn when handling frozen vials. Some leakage may occur into the vial during storage. The liquid nitrogen will be converted to gas upon thawing. Due to the nature of nitrogen gas, pressure may build within the vial and possibly result in the vial exploding or losing its cap. This may cause flying debris.

Thawing procedure: The vial of cells should be thawed in a 37 °C water bath with gentle agitation. For optimal performance, the vial should be thawed in under two minutes. Ensure that the cap of the vial did not loosen upon thawing, and re-tighten as needed. Spray the vial with 70% EtOH and wipe off. Repeat. Using aseptic technique, add the contents of the vial to 9 ml of complete growth medium (without selection). Centrifuge for 5 min. at 125 x g. Aspirate the medium, being careful not to disturb the pellet. Resuspend in 10 mL of complete growth medium, and place into a culture vessel of your choice. Only add selection to the medium after 24 hours in culture.

Culture conditions:

Complete growth medium

The base medium for this cell line is DMEM. For optimal growth and maintenance of selection, add the following components to the base medium: dialyzed fetal bovine serum to a final concentration of 10%.

Selection: Hygromycin to a final concentration of 100 µg/mL
Puromycin to a final concentration of 1 µg/mL

Culture temperature: 37 °C with 5% CO₂

Subculture: Replace culture medium with selection-free medium and incubate for up to 6 hours. Rinse the cells with PBS without cations, digest cells with 0.25% (w/v) Trypsin-EDTA (0.53 mM) solution and split at 1:3 to 1:10 ratio.

Cryopreservation: CultureSure Freezing Medium (WAKO, Cat# 039-23511, 100 mL)
Optional: Freeze slowly in complete growth medium supplemented with 10% (v/v) DMSO.

Mycoplasma: Negative
(MycoAllert Mycoplasma Detection Kit from Lonza)

Product QC: >95% viability before freezing. All cells were tested and found to be free of mycoplasma, bacterial, viruses, and other toxins.

Tips for handling:

- a. When changing the medium, retain 1/5 of the old medium, and add 4/5 fresh medium to reduce the floating of cells during fluid exchange.
- b. To prevent SL222 cells from detaching in 96 well plates, coat the bottom of each well of a 96 well plate with 0.1% gelatin (50ul/well) and incubate 30min at 37°C. Remove the gelatin and plates are ready to use. (Optional: Poly-D-lysine can also be used for coating)

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