Product Specification Sheet

Product Name	**HEK293-hACE2 Cell Line**
Description	Human angiotensin-converting enzyme 2 (hACE2) is an enzyme on the cell membranes of cell in multiple organs such as lungs, heart, kidney, arteries and intestines. It serves as a critical entry receptor for many coronaviruses, including SARS-CoV, MERS-CoV, as well as SARS-CoV-2 that causes COVID-19, a pandemic disease. This HEK293-hACE2 cell line is derived from HEK293 cells by transducing lentivirus particles encoding human ACE2 gene. The cell line stably expresses hACE2 and is an excellent cell resource for in vitro drug screening and characterization against coronaviruses includingSARS-CoV-2 virus.
Catalog Number	AL01
Size	1 x10^6 cells/vial
Shipping	Dry ice
Storage and Stability	Store in vapor phase of liquid nitrogen immediately upon receipt. This product is stable for 6 months when stored as directed.
Quality Control	Each vial contains about 1 x 10^6 cells with >95% viability before freezing. Each lot of cells is tested for infectivity of spike (SARS-CoV-2) pseudotyped GFP lentivirus, growth and viability following recovery from cryopreservation, and free of mycoplasma and competent lentivirus as well.
Safety Precaution	**ALSTEM highly recommends that protective gloves, a lab coat, and a full-face mask are always worn when handling frozen vials.** It is important to note that some liquid nitrogen can leak into the vials when submersed in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in excessive pressure within the vial that can cause the vial to explode or expel the cap with dangerous force.
Restricted Use	For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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HEK293-hACE2 Cell Line

Product Descriptions

Human angiotensin-converting enzyme 2 (hACE2) is an enzyme on the cell membranes of cell in multiple organs such as lungs, heart, kidney, arteries and intestines. It serves as a critical entry receptor for many coronaviruses, including SARS-CoV, MERS-CoV, as well as SARS-CoV-2 that causes COVID-19.

This HEK293-hACE2 cell line is derived from HEK293 cells by transducing lentivirus particles encoding human ACE2 gene. The cell line stably expresses hACE2 and is an excellent cell resource for in vitro drug screening and characterization against coronaviruses including SARS-CoV-2 virus.

Characterization:

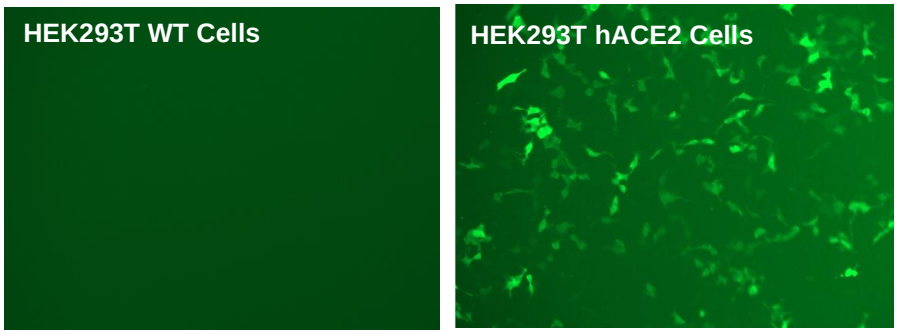

Figure 1. Infection of HEK293-hACE2 cells by pseudotyped GFP SARS-CoV-2 virus (Cat# SV01). GFP expression was observed on infected hACE2 cells 48 hours post transduction, wherease no GFP expression was detected in HEK293 wild type (WT) cells.

Culture Protocol:

Preparation of culture medium

Growth Medium:
90% D-MEM with GlutaMAX™-I (high glucose)
+ 10% Fetal bovine serum (FBS)
+ 1% Penn/Strep

Freezing Medium:
70% D-MEM with GlutaMAX™-I (high glucose)
+20% Fetal bovine serum (FBS)
+ 10% DMSO

Thawing HEK293-hACE2 cells

1. **Day 1**: Place 10 mL of Growth Medium into a T-75 flask.

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2. Place the flask in a humidified 37 ºC / 5% CO_2 incubator for 15 min to allow medium to equilibrate to the proper pH and temperature.
3. Remove the vial of cells from liquid nitrogen and rapidly thaw by placing at 37 ºC in a water bath with gentle agitation for 1–2 min. (Do not submerge vial in water.)
4. Decontaminate the vial by wiping with 70% ethanol before opening in a Class II biological safety cabinet.
5. Transfer the vial contents drop-wise into 10 mL of Growth Medium in a sterile 15-mL conical tube.
6. Centrifuge cells at 200 × g for 5 min.
7. Aspirate supernatant and resuspend the cell pellet in 1 mL of fresh Growth Medium.
8. Transfer contents to the T-75 tissue culture flask containing pre-equilibrated Growth Medium and place flask in a humidified 37 ºC / 5% CO_2 incubator.
9. Change Growth Medium every day or when pH decreases. Monitor the cells every day.

Passage of HEK293 –hACE2 cells

Cells are split when they reach about 85% confluence.

1. Pre-warm fresh Growth Medium in fresh culture vessel in incubator.
2. Aspirate medium from growing cells, rinse once in PBS, add 0.05% Trypsin/EDTA (5 mL for a T-75 flask, 10 mL for a T-175 flask) and swirl to evenly coat the cells. Incubate for 3min. After 3 min, tap the flask firmly on the side. Cells usually detach easily.
3. Add an equal volume of Growth Medium (5 mL for a T-75 flask, 10 mL for a T-175 flask) to inactivate Trypsin/EDTA, and break up the cell clumps by gently pipetting up and down several times.
4. Verify under a microscope that cells have detached and clumps have completely dispersed.
5. Count the cell number using a hemocytometer. Dilute cells 1:1 with 0.4% trypan blue. Add 10 ul to a hemocytometer and count the cells. *Count at least 4 quandrants.*
6. Centrifuge cells at 200 × g for 5 min and resuspend in Growth Medium.
7. Plate $2x10^6$ cells per T-175 flask with 25 mL of Growth Medium.
8. Incubate at a humidified 37 ºC / 5% CO_2 incubator.
9. Change media the day after plating and every 3 days, until the cells reach 85% confluence. Monitor the cells every day. Add puromycin to the medium to final concentration of 1 µg/mL after 24 hours in culture.

Freezing HEK293-hACE2 cells

1. Aspirate the medium and wash the cells with 25 ml of warm DPBS.
2. Aspirate DPBS, and add 10 ml of 0.05% trypsin-EDTA, and incubate for 2-3 min. After 3 min, tap the flask firmly on the side. The cells should be observed coming off the flask easily.
3. Add 10 ml of Growth Medium and break up the cell clumps by gently pipetting up and down several times.
4. Transfer cells to a conical tube, count the number of cells using 0.4% trypan blue, and centrifuge at 200 g for 5 min.
5. Discard the supernatant, resuspend the pellet in cold Freezing Media to a density of $2x10^6$ cells/mL, and aliquot in 1 ml/cryovial.
6. Place the vials in a Mr. Frosty cell-freezing container and keep it at -80 °C overnight.
7. Transfer the vials to a liquid nitrogen tank for long-term storage.

IMPORTANT NOTICE

Store the vials at vapor phase of liquid nitrogen immediately upon receipt.

WARNING

Do not use cryogenic vials for storage in the liquid phase of liquid nitrogen. Such use may cause entrapment of liquid nitrogen inside the vial and lead to pressure buildup resulting in possible explosion or biohazard release. Use appropriate safety procedures that are outlined by the ATCC when handling and disposing of vials. ALSTEM highly recommends that protective gloves and clothing always be used and a full-ace mask always be worn when handling frozen vials.

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