I. Notes

The Human ACE2 293T Cell Line (Cat. No. 631289) should be cultured immediately upon receipt, or as soon as possible thereafter. Significant delays in culturing may result in decreased cell viability. **We recommend using collagen-coated plates or flasks for efficient culturing of this cell line.** Vessels coated with compounds other than collagen may also provide suitable growth substrates (e.g., poly-l-lysine), but only collagen has been tested at Takara Bio.

Expressing a constant level of ACE2 at the cell surface, this cell line can be used in conjunction with pseudovirus generated using Lenti-X™ SARS-CoV-2 Packaging Single Shots for the identification of factors that mediate or inhibit infection of host cells (e.g., by performing neutralization assays), and in the study of interactions between the SARS-CoV-2 spike protein and ACE2 receptor.

II. Starting Human ACE2 293T Cell Line cultures from frozen stock:

To prevent osmotic shock and to maximize cell survival, perform the following:

1. Warm ~25 ml of complete culture medium (90% Dulbecco's Modified Eagle's Medium [DMEM] with high glucose (4.5 g/L), 4 mM L-glutamine, and sodium bicarbonate; 10% Fetal Bovine Serum (FBS); and 1 mM sodium pyruvate) in a 37°C water bath.

2. Thaw vial of cells rapidly in a 37°C water bath with gentle agitation. Immediately upon thawing, remove from the water bath and wipe the outside of the vial with 70% ethanol.

   NOTE: All steps from this point on should be carried out in a laminar flow tissue culture hood under strictly aseptic conditions.

3. Add 1 ml of the prewarmed medium to a 15-ml conical centrifuge tube.

4. Unscrew the top of the vial of cells slowly. Using a narrow pipet, transfer the contents of the vial to the 15-ml conical centrifuge tube containing 1 ml of prewarmed medium. Mix gently.

5. Slowly add an additional 4 ml of fresh, prewarmed medium to the tube. Mix gently.

6. Add an additional 5 ml of fresh, prewarmed medium to the tube. Mix gently. Centrifuge at 100*g* for 5 min. Carefully aspirate the supernatant, and **gently** resuspend the cells in complete medium without selective antibiotics.

   NOTE: This method removes the cryopreservative and can be beneficial when resuspending in small volumes. However, be sure to treat the cells gently to prevent damaging fragile cell membranes.

7. Mix the cell suspension thoroughly and add to a suitable culture vessel. Gently rock or swirl the dish/flask to distribute the cells evenly over the growth surface and place it in a humidified 37°C incubator (5–10% CO_2 as appropriate) for 24 hr.

8. After incubation, examine the cells under a microscope. If the cells are well-attached and confluent, they can be passaged for immediate use. If the majority of cells are not well-attached, continue culturing for another 24 hr. Complete attachment of newly thawed cultures of HEK 293-based cell lines may require incubation up to 48 hr.

III. Freezing the Human ACE2 293T Cell Line

Once the culture has been started and the cells are growing normally, prepare frozen aliquots to provide a renewable source of cells.

1. Trypsinize the desired number of flasks or plates.
2. Pool cell suspensions together, count the cells, and calculate the total viable cell number.
3. Centrifuge cells at 100*g* for 5 min. Aspirate the supernatant.
4. Resuspend the pellet at a density of at least 1–2 x 10^6 viable cells/ml in freezing medium (e.g., Sigma-Aldrich Cat. No. C6164; or 70–90% FBS, 0–20% medium, and 10% DMSO).
5. Dispense 1-ml aliquots into sterile cryovials.
6. Freeze slowly (1°C per min). To achieve this, place the vials in cryogenic freezing containers (e.g., Nalgene Cat. No. 5100) and freeze at –80°C overnight. Alternatively, place vials in a thick-walled styrofoam container at –20°C for 1–2 hr. Then, transfer to –80°C and freeze overnight. Remove vials from the freezing containers the following day, and store in liquid nitrogen or an ultra-low temperature freezer (–150°C).
7. Two or more weeks later, plate a vial of frozen cells to confirm viability.

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This document has been reviewed and approved by the Quality Department.