Human Foreskin Fibroblast Cell Lines (Neonatal and pooled)

Cat.# PC501A/B-HFF

User Manual

Store kit at -80°C on receipt

ver. 091012-1

Contents

List of Components

The Human Foreskin Fibroblasts, neonatal (HFFn), are available as cryopreserved cells in one vial. PC501A-HFF is optimized for the usage as iPS source cells, whereas PC502B-HFF is optimized for the usage as feeder cells.

PC501A-1	HFFn, One Vial	$1x10^6$ cells
PC502B-1	HFFn, One Vial	$1x10^6$ cells

The cryopreserved HFFn cells are shipped on dry ice and should be immediately stored in liquid nitrogen upon receipt. Properly stored cells are stable for more than 2 years from the date received.

I Human Foreskin Fibroblasts, neonatal

A. Description

Human foreskin fibroblasts, neonatal (HFFn, cat# PC501A-HFF and PC502B-HFF) were isolated from neonatal foreskin. Each vial of this product contains $1X10^6$ viable cells. In our laboratory, each lot of cells is performance tested by culturing the cells through multiple passages in the absence of antibiotics and antimycotics, no contamination was observed during this culture period. Upon thawing, the cells are guaranteed to be >75% viable and have a potential of >30 population doublings when cultured according to the instructions provided in this manual.

B. Maintenance of HFFn Cultures

Initiating Cultures from Cryopreserved Cells

To insure the highest level of viability, be sure to warm medium to 37 °C before using it on the cells. We recommend seeding cells recovered from cryoperservation at a cell density of 4×10^3 viable cells/cm^2. The procedure given below is a sample protocol for establishing cultures from the contents of one vial.

1. Prepare a bottle of DMEM supplemented with 10% heat-inactivated FBS with/without antibiotics.
2. Remove the vial from liquid nitrogen storage, taking care to protect hands and eyes.
3. Dip the lower half of the vial into a 37°C water bath to thaw.
4. When the contents of the vial have thawed, wipe the outside of the vial with 75% alcohol to disinfect and move the vial to a laminar flow culture hood.
5. Open the vial and pipet the cell suspension up and transfer the cells into a 15-cm conical tube with 10 ml fresh culture medium.
6. Pipet up and down with a 10 ml pipette to disperse the cells and centrifuge the cells at 180 g for 5 minutes. Observe the cell pellet.
7. Remove the supernatant form the tube, being careful not to dislodge the cell pellet.
8. Resuspend the cell pellet with fresh culture medium and seed new culture vessels with 4×10^3 cells/cm^2.
9. Incubate the cultures in a 37 °C, 5% CO_2 /95% airhumidified cell culture incubator.
10. Do not disturb the culture for at least 24 hours after the culture has been initiated.

Maintaining Stock Cultures

1. Change the culture medium 24 to 36 hours after establishing the secondary culture from cryopreserved cells.
2. Change the medium every other day thereafter, until the culture is approximately 80~90% confluent.

Subculturing HFFn

1. Remove all of the culture medium from the T75 flask.
2. Washing cells with 10 ml pre-warmed DPBS once.
3. Add 3 ml of 0.25% trypsin-EDTA solution to the flask. Rock the flask to ensure that the entire surfaced is covered.
4. Incubate the flask at room temperature until the cells have become round, approximately 2~4 minutes. View the culture frequently under a microscope to avoid over digestion.
5. Add 7 ml of culture medium to the flask and transfer the detached cells to a sterile 15 ml conical tube.
6. Centrifuge the cells at 180 g for 5 minutes.
7. Remove the supernatant from the tube, being careful not to dislodge the cell pellet.

8. Resuspend the cells pellet in 10 ml supplemented medium. Pipet the cells up and down with a 10 ml pipette to ensure a homogeneous cell suspension.
9. Determine the concentration of cells in the suspension.
10. Seed new culture vessels with 4 x 10^3 cells/cm^2, or 1 to 3~5 split if starting cells at a 90% confluence.
11. Incubate the cultures in a 37 °C, 5% CO_2 /95% air humidified cell culture incubator.

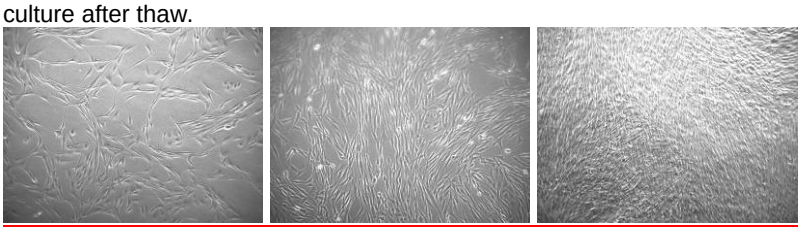
Cryopreserving HFFn

1. Follow steps 1-6 from the Subculturing of Cells above.
2. Resuspend the cell pellet in culture medium. Add approximately 1 ml for each T75 flask.
3. Count the number of cells and dilute the cell suspension to 2 x 10^6 cells/ml.
4. Add an equal volume of cold 2X Freezing Media to the cell suspension.
5. Aliquot 1 ml of suspension into each cryovial (1 x 10^6 cells/vial).
6. Place the vials in a cell-freezing container and keep it at -80 °C overnight.
7. Transfer the vials to a liquid nitrogen tank for long-term storage.

Note: We recommend to use DMEM supplement with 20% FBS and 10% DMSO as freezing media to cryopreserve HFF cells.

C. Morphology of HFFn in Culture

HFFn cells grown in DMEM supplemented with 10% FBS without antibiotics. Images of HFFn in culture after thaw.



Day1 –HFFn **Day 3 –HFFn** **Day 5 -HFFn**

D. References

Takahashi, K. and Yamanaka, S. 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126: 663–676.

Takahashi K. et al. 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 131: 861–72.

Park, IH et al. 2008. Reprogramming of human somatic cells to pluripotency with defined factors. Nature. 451:141–6.

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For more information about SBI products and to download manuals in PDF format, please visit our web site:

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