SOP: Propagation of PGP1

ENCODE4 - Version 1

Information

Name: PGP1
Organism: Homo sapiens, Human
Tissue: Fibroblast derived iPS cells, 53-year-old Caucasian male
Culture Properties: adherent
Biosafety Level: 1

Material List

1. StemMACS ips-Brew XF Basal medium plus supplement (Miltenyl Biotech; Cat # 130-104-368)
2. mTeSR1 complete kit (basal medium plus supplement) (Stem Cell Technology, Cat # 85850)
3. Penicillin-Streptomycin 10,000U (Life Technologies, Cat # 15140 or Corning Cellgro, Cat # 300-002-CI)
4. Phosphate Buffered Saline (1X PBS) w/o Ca^{2+}, Mg^{2+} (CORNING Cellgro; Cat # 21-040-CM)
5. 0.5 M EDTA, pH8.0 (ThermoFisher Scienfic; Cat # 15575020)
6. Accutase (ThermoFisher Scientific; Cat # A11105-01)
7. Y27632 (Fisher Scientific; Cat # 50-863-6)
8. Matrigel (CORNING; Cat # 354277)
9. Tissue culture treated 6 well plate and 100x20mm dish
10. Graduated pipets (2, 5, 10, 25, 50 ml)
11. 2x Cryopreservation medium (GE Healthcare, Cat # SR30002.02)
12. Coating medium: DMEM/F12 (CORNING Cellgro; Cat # 10-092-CV)
13. Cryovials (Sarstedt; Cat # 72-694-006)
14. TC20 cell counter (Bio-Rad)
15. Counting Slides (Bio-Rad; Cat # 145-0011)
16. 0.40% Trypan Blue Dye (Bio-Rad; Cat # 145-0013)
17. Microscope

Growth Medium for PGP1

mTeSR complete medium: 400 ml basal medium + 100 ml 5 x supplement + 5 ml 100x Pen-Strep
StemMACS complete medium: 500 ml basal medium + 10 ml 50x supplement + 5 ml 100x Pen-Step

- Before use let the medium warm up to RT.
- Complete medium is good to use for 1 week, if can not finish the medium in one week, suggest aliquot basal medium (save at 4°C) and supplement (save at -20°C), prepare enough medium for use in one week.

Procedure

A. Plate coating for PGP1

Note: Coating is necessary because otherwise PGP1 cells can't stick to the plates.

Coating solution: Matrigel (stored at -80°C) (See Matrigel instruction for aliquot and handling)
Coating medium: DMEM/F12 (stored at 4°C)

1) In the hood put 25 ml coating medium into a 50 ml tube.
2) Take coating solution from -80°C freezer.
3) Transfer about 500 µl coating medium from 50 ml tube to coating solution tube. Pipet to thaw coating solution and transfer back to 50 ml medium tube. Repeat until all coating solution thawed and was transferred to 50 ml tube with coating medium.
4) You will need following volumes:
    a) 1 ml need for one well on a 6-well plate
    b) 0.5 ml need for one well on a 12-well plate
    c) 6 ml need for one 10cm dish
5) Coated plate can be used after overnight incubation at 4°C or 3-4 hours at RT or at least 1h at 37°C.
6) Coated plates can be stored at 4°C for up to one week.

B. Receipt of Frozen Cells and Starting Cell Culture

1) Immediately place frozen cells in liquid nitrogen freezer storage until ready to culture.
2) When ready to start cell culture, quickly thaw ampoule in a 37°C water bath.

3) As soon as ice crystals disappear, swab outside surface of the ampoule with 70% ethanol, then dispense contents of ampoule into a 15 ml tube with 10 ml of growth media.
4) Centrifuge cells at 1000 rpm for 4 minutes at RT.
5) Remove supernatant, resuspend cells with 2-3 ml growth media with Y27632 (1:1000 diluted) with gently pipetting for 4-5 times and transfer cells to pre-coated 1-2 wells of 6-well plate.
6) Allow cells to recover overnight in 37°C, 5% CO2 humidified incubator.
7) The next morning, change fresh medium.

C. Sub-culture

1) Propagate cells until density reaches 70-80% confluence.
2) Remove and discard culture medium.
3) Briefly rinse cell layer with room temperature 1X PBS and remove PBS.
4) Add 1 ml EDTA (0.5mM in PBS) (RT store) to one well of 6-well plate (0.5 ml to one well of 12-well plate, or 3-4 ml to one dish), incubate at RT for 5-10 min or until cells almost detach. Do NOT let the cells detach. Do NOT shake the plates or dish.
5) Remove EDTA, wait for 1 min and add 2 ml medium to one well of 6-well plate to suspend cells using 5 ml pipet.
6) Pipet 5-6 times and transfer ¼ -⅛ cells to new coated wells, 2-4 ml medium total in each well. Gently shake plates horizontally to evenly spread the cells.
7) Incubate cultures at 37°C, 5% CO_2 humidified incubator.
8) Record each subculture event as a passage.

D. Maintenance and Generation of Seed Stocks

1) Change media EVERYDAY thereafter. Use 2-4 ml (one well pf 6-well plate) or 1-2 ml (one well of 12-well plate) or 10-20 ml (10cm dish) of growth medium.
2) Remove differentiated cells before changing medium whenever they appear.
3) Following first or second passage after receipt of cells and with sufficient number of cells to continue maintenance and expansion, the major portion of the wells should be sub-cultured using EDTA as above under “Sub-culture” and a small portion should be set aside as a seed stock.
4) The cells for the seed stock should be re-suspended in 1x Cryopreservation medium (mixture of same amount of culture medium with 2x Cryopreservation medium) after EDTA treatment. One well of 6-well plate cells can be resuspended in 1-2 ml 1x Cryopreservation medium.

5) Cells in freezing medium are dispensed into cryovials (1 ml aliquot/cryovial) and frozen in a -80^{o}C cryo-freezing container overnight.
6) Cryovials are transferred the next day to liquid nitrogen freezer for long-term storage.

E. Harvest

1) Remove and discard culture medium.
2) Briefly rinse cell layer with room temperature 1X PBS and remove PBS.
3) Add 1 ml Accutase to one well of 6-well plate (0.5 ml to one well of 12-well plate, or 3-4 ml to one dish), incubate at 37^{o}C for 2-3 mins or until cells detach.
4) Add 2 ml (6-well plate) or 1 ml (12 well plate) or 4-5 ml (10 cm dish) of complete growth medium and aspirate the cells by gentle pipetting. Transfer the cell suspension to the centrifuge tube.
5) Examine viability using Trypan blue staining and count cells. Transfer desired amount of cells to new tube and centrifuge and wash cells with PBS once for your application if needed.