WTC11 iPS cells culture protocol (TIER 2)

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Maintaining Feeder-Free iPS Cells (iPSC) in E8 Media

Table 1. Volume for each vessel format

Vessel formats	Vendor	Cat #	Vol. (ml/well) for Matrigel coating	Vol. (ml/well) for media exchange	Surface area (cm^2/well)
15 cm dish	Corning	430599	15 ml	25 ml	148
10 cm dish	Nunc	174902	5-7 ml	10 ml	56.7
6 well plate	BD Science	351146	1.5 ml	2 ml	9.6
12 well plate	BD Science	351143	1 ml	1 ml	3.8
24 well plate	BD Science	351147	0.5 ml	0.5 ml	2
48 well plate	BD Science	351178	0.25 ml	0.25 ml	0.75
96 well plate	BD Science	351172	0.125 ml	0.125 ml	0.32
T25 flask			4 ml	5 ml	25
T75 flask			12 ml	15ml	75

Reagents:

GFR Matrigel Phenol Red Free (BD Bioscience/Corning)
Essential 8™ Medium (prototype), #A14666SA, (Life Technologies)
Y-27632 ROCK inhibitor (Ri) #688000, 10 mM stock (EMD)
Accutase (Stemcell Technologies)

80 µg/ml GFR Matrigel medium:

500 µl of 4°C cold 8 mg/mL GFR Matrigel Phenol Red-Free (BD Bioscience)
50 ml of 4°C cold Knockout (KO) DMEM #10829-018 (Life Technologies)

iPSC Freezing media:

10% DMSO in 90% FBS (+) Ri (10 µM final concentration; *optional*)
-> filter sterilize. This media is good for a month at 4°C

A. Thawing iPSC

1. Coat a T25 flask with 1.5 ml/well of 80 µg/ml GFR Matrigel medium overnight in the 37°C incubator (1 day prior to cell seeding).
2. Get a vial of frozen cells from the nitrogen tank.
3. Prepare a 15 ml conical tube with 2 ml of E8/Ri.
4. Thaw the vial of frozen cells by warming quickly in your hands, or in the 37ºC incubator.
5. When the cells are about almost completely thawed, transfer the cell suspension to the 2 ml of E8/Ri in the 15 ml tube. Rinse the cryotube with the same medium to collect the remaining cells.
6. Mix the cell suspension 2-3 times.
7. Spin the cell suspension at 800 rpm for 3 min.
8. Aspirate the supernatant and resuspend the pellet with 5 ml E8/Ri medium.
9. Let the cells sit in the medium for 5 min, and then plate in the Matrigel-coated T25 flask.
10. After 24 h, add 5 ml of E8 medium.
11. Feed the cells everyday with E8.

B. iPSC passaging in T25 flask format

-> adjust volumes accordingly, depending on the vessel format

1. Coat a T25 flask with 4 ml of Matrigel medium overnight in the 37°C incubator (1 day prior to cell seeding).
2. Split the cells at 70-80% confluency.
3. Wash the iPS cells 1X with PBS.
4. Add 1.5 ml of Accutase to the cells.
5. Incubate cells in 37°C incubator for 3 min.
6. Check the cells for detachment by knocking the flask against your palm.
7. Add 10 ml of PBS to the cells to dilute down the Accutase.
8. Pipette up and down a few time for dissociation.
9. Centrifuge the cells at 800 rpm for 3 min.
10. Resuspend the pellet with 3ml of E8 (+) 10 µM Ri medium.
11. Let the cells sit at room temperature for 5 -10 min in the hood.
12. Pipette up and down to singularize the cells.
13. Prepare Countess slide for counting: ** (see below).*
    - add 10 µl of singularized cell suspension and 10 µl of Trypan blue to an eppendorf tube.
    - Pipette up and down a few times for further dissociation.
    - take a 10 µl aliquot of the cells and inject into a chamber of the Countess slide.
    - use iPSC program to count live cells: make sure there are less than 5 cells excluded, marked by black rings, by pressing the Zoom In button. *If there are many black rings shown, go back to the original cell suspension, pipette up and down for further dissociation, and prepare a new aliquot for re-counting.*

19. Aspirate any excess Matrigel liquid from the flask.
20. Add 5 ml of E8/Ri medium in the flask.
21. For WTC11, transfer 2E5 cells into the flask. *For other iPS cell lines, try 2E5 to 4E5 for a T25 flask.*
22. Rock the flask in an East/South/West/North direction and incubate the cells at 37°C.
24. The cells should be at 70-80% confluency on day 4.

** Optional: it is also possible to not count the cells and perform a ratio split, e.g. 1:15, 1:20 split of iPSC:media, and plate. However, if the iPSC are required at a specific time, it is better to count and plate a defined number of iPSCs for greater accuracy.*

C. Freezing iPSC

-> adjust volumes accordingly, depending on the vessel format

1. Freeze the cells at 70% - 80% confluency.
2. Wash the iPS cells 1X with PBS.
3. Add 1.5 ml of Accutase to the cells in T25 flask.
4. Leave the accutased cells in 37°C incubator for 3 min.
5. Check the cells for detachment by hitting the flask against your palm.
6. For T25 flask, dilute the accutase with 10 ml of PBS.
7. Pipette up and down a few times to dissociate the cells.
8. Centrifuge the cells at 800 rpm for 3 min.
9. Resuspend the pellet in the freezing medium.
    * For a T25 flask, freeze the cells in 3 aliquots into cryovials.
10. Put the cryotubes into a room temperature Mr. Frosty and place in the -80°C freezer.
11. Transfer the cells from -80°C to the liquid nitrogen tank the next day.