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iPSC Cell Culture – Maintenance and Expansion

Jacob Marsh[1], Celeste Karch[2]

[1]Washington University in Saint Louis - WUSTL (MO), [2]Washington University in St Louis

1 Works for me dx.doi.org/10.17504/protocols.io.9pwh5pe

Neurodegeneration Method Development Community

ABSTRACT

This protocol is about Maintenance and Expansion of induced pluripotent stem cells.

ATTACHMENTS

iPSC_Cell_Culture_Maintenance_and_Expansion_112019_2.pdf

MATERIALS TEXT

- DMEM/F12 medium
- Matrigel
- mTesR1 media
- Rock Inhibitor
- 1x PBS
- Accutase (Gibco A11105-01)

StemPro™ Accutase™ Cell Dissociation Reagent
by Thermo Fisher Scientific
Catalog #: A1110501

- 2X Freezing Medium (20 % DMXO, FBS)

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

BEFORE STARTING

Warm all media before use. Media should be **Room temperature** . Do **not use a water bath** to warm media.

Matrigel Coating

1 To resuspend, thaw aliquot **On ice** .

2 Add **12.5 ml** cold DMEM/F12.

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3 

Pipette up and down twice.

4 

Add **1 ml** of Matrigel per well of 6 well plate.

5 Store diluted Matrigel at **4 °C** .

Thawing iPSC

6 Prior to thawing cells, coat plate with Matrigel for **01:00:00** .

1 vial of iPSC should be thawed into 1 well of a 6 well plate.

7 

Add **9 ml** DMEM/F12 to a 15 ml conical tube labeled with the iPSC line name and passage number.

8 Remove cells from liquid nitrogen storage.

9 Quickly thaw cells in **37 °C** water bath and/or in hands.

10 Just prior to complete thaw, remove vial from water bath.

11 

Transfer the contents of the cryo-vial (~ **1 ml**) into the 15 ml conical tube.

12 

Spin at **750 rpm** for **00:03:00** at **Room temperature** .

13 Aspirate media.

14 

Resuspend cells in **2 ml** mTesR1 (supplemented with **5 Micromolar (µM)** – **10 Micromolar (µM)** Rock Inhibitor) by **pipetting two times**.

15 

Transfer the cell solution to one well of a 6-well plate.

16 

Incubate at **37 °C** **Overnight** in **6 %** CO_2.

17 **Replace** the **media daily** until cells are ready to split or analyze.

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Cell Growth/Maintenance

18

Media should be changed daily. It is okay to skip a media change one time each week if double feeding is performed; however, this is largely dependent on the density of the cells and volume of media (do **not double feed** if cells are **more than 70% confluent**).

Aspirate media.

19

Gently add fresh mTesR1 to cells (volume depends on cell density and well size).

- **0.5 ml** per well to 24 well plate
- **2 ml** — **4 ml** per well to 6 well plate
- **5 ml** — **10 ml** to 10 cm^2 plate

20

Incubate at **37 °C** in **6 % CO_2.**

Cleaning

21

When differentiating cells appear in the culture, it is important to remove all the cells promptly.
Repeated cleaning may be necessary over the course of several days to remove all the material. If differentiation is excessive and line is precious, perform subcloning.

Under microscope, remove differentiated cells with p20 or p200 tip (depending on the amount of differentiation). Transfer the cells/media to a biohazard bag.

22

Gently wash cells with 1x PBS.

23

Add fresh mTesR1.

- **0.5 ml** per well to 24 well plate
- **2 ml** — **4 ml** per well to 6 well plate
- **5 ml** — **10 ml** to 10 cm^2 plate

24

Incubate at **37 °C** in **6 % CO_2** until cells are **60 — 80 % confluent**. Change mTesR1 media **daily** until cells are needed. Repeat cleaning as necessary.

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Splitting/Expanding

25 

iPSCs grow on Matrigel. Plates should be coated with Matrigel at least 1 hour prior to plating and no longer than 24 hours prior to plating cells:

- **0.5 ml** in 12 well plate
- **1 ml** in 6 well plate
- **4 ml** in 10 cm^2 plate

It is critical to keep Matrigel on ice while coating. Prior to plating cells, ensure Matrigel has not evaporated from well.

26 Aspirate media.

27 

Gently wash cells with 1x PBS (**2 — 3 ml/well**).

28 

Add Accutase (Gibco A11105-01) directly to the cells and incubate at **37 °C** for **00:03:00** — **00:04:00** .

- 6 well plate, add **0.75 ml** — **1 ml** per well
- 24 well plate, add **0.5 ml**
- 10 cm^2 dish, add **3 ml**

29 Tap dish to aid in dislocation of cells.

30 

Add DMEM/F12 directly to cells and scrape gently to remove all cells (use p1000 for 24 well plate, and cell scraper for 6 well plate and 10cm^2 dish).

- 6 well plate, add **2 ml** — **4 ml** per well
- 24 well plate, add **1 ml**
- 10 cm^2 dish, add **9 ml**

31 Collect cells in conical tube (15 ml/50 ml depending on volume).

32 

If necessary, add **2 ml** — **5 ml** DMEM/F12 to dish to remove all cells from the dish and add to conical tube.

33 

Centrifuge cells at **750 rpm** for **00:03:00** at **Room temperature** .

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34

Carefully aspirate supernatant.

To avoid aspirating cell pellet, it is OK to leave a small amount of media (**0.5 ml** — **1 ml**).

35

Resuspend cell pellet with mTesR1 (Rock Inhibitor addition varies, see below).

- **2 ml** mTesR1 per well of a 6 well plate
- Our goal is to maintain iPSC lines without using Rock Inhibitor; however, this must be done through careful weaning off Rock Inhibitor
- All cells should be thawed in Rock Inhibitor:
  - **10 Micromolar (µM)** concentration for new iPSC lines, lines thawed from 96 well after editing.
  - **5 Micromolar (µM)** concentration if thawing from a line without knowledge of its Rock sensitivity.
  - **1 Micromolar (µM)** concentration for all other lines (for lines still exposed to Rock Inhibitor, use **1 Micromolar (µM)** . Otherwise, do not use Rock Inhibitor.)

36

Pipet cells **2 times** only to preserve clumps.

37

Transfer cell suspension to appropriate plate (pre-coated with Matrigel for at least **01:00:00**).

- For maintenance, dilute cells 1:3 in mTesR1
- For expansion, plate all cells

38

Incubate at **37 °C** in **6 % CO_2** until cells are **60 — 80% confluent**. Change mTesR1 media **daily** until cells are needed.

iPSC Freezing

39 Aspirate media.

40

Gently wash cells with 1x PBS (Use **2 ml** — **3 ml** per well in 6 well plate).

41

Add Accutase (Gibbco A11105-01) directly to the cells and incubate at **37 °C** for **00:03:00** — **00:04:00** .

- 6 well plate, add **0.75 ml** — **1 ml** per well
- 10cm^2 dish, add **3 ml**

42 Tap dish to aid in dislocation of cells.

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43

Add DMEM/F12 directly to cells.

- 6 well plate, add **2 ml** — **4 ml** per well
- 10cm^2 dish, add **9 ml**
- If cells remain attached, use a cell scraper to gently dislodge cells (apply gentle pressure and use 1 — 2 passes to remove cells)

44 Collect cells in conical tube (15 ml/50 ml depending on volume).

45

Add **2 ml** — **5 ml** DMEM/F12 to dish to remove all cells from the dish and add to conical tube.

46

Centrifuge cells at **750 rpm** for **00:03:00** at **Room temperature** .

47

Carefully aspirate supernatant.

To avoid aspirating cell pellet, it is OK to leave a small amount of media (**0.5 ml** — **1 ml**).

48

Resuspend cell pellet with mTesR1 (No Rock Inhibitor).

- Use volume appropriate for freezing
- Assume **1 ml** per cryovial total and add ½ total volume of mTesR1
- Pipet cells 1 — 2 times only to preserve cell clumps

Example: to freeze 10 tubes, you will need **10 ml** total and will add **5 ml** mTesR1 to cell pellet (and **5 ml** of 2x Freezing Media below)

49

Add an equal volume of cold 2x Freezing Media (20 % DMSO, FBS). Pipet cells 1 time only to preserve cell clumps.

50

Transfer cell suspension to pre-labeled cryovials (**1 ml** per cryovial).

Ensure that cryovials are labeled with the following:

- Cell Type
- Line Name
- Passage #
- Date
- Your Name

51 Freeze vials at **-80 °C** in foam racks for **48:00:00** — **72:00:00** .

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52 Transfer vials to **liquid nitrogen** for long-term storage.

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