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iPSCs Maintenance and Banking V.2

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We use this protocol and it's working

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Abstract

This protocols offers a thorough description of the thawing, maintenance and banking of human induced pluripotent stem cells.

Materials

Matrigel:

Matrigel catalog number: **Cornig 354234**
Matrigel stock concentration: **9-11 µg/µL**
Matrigel working concentration: **90-110 µg/mL (1:100 dilution)**
Matrigel Stock aliquot volume: **400 µL**

Culture Antibiotic:

Primocin catalog number: **InvivoGen ant-pm-1**
Primocin stock concentration: **50 mg/mL**
Primocin working concentration: **100 µg/mL**
Primocin stock aliquot volume: **1 mL**

CEPT:

Chroman 1 catalog number: **MedChemExpress HY-15392**
Chroman 1 stock concentration: **50 µM**
Chroman 1 working concentration: **50nM**

Emricasan catalog number: **SelleckChem S7775**
Emricasan stock concentration: **5 mM**
Emricasan working concentration: **5 µM**

Polyamine supplement catalog number: **SigmaAldrich P8483**
Polyamine supplement working concentration: **1000x**
Polyamine supplement stock concentration: **1x**

Trans-ISRIB catalog number: **R&D systems 5284**
Trans-ISRIB stock concentration: **700 µM**
Trans-ISRIB working concentration: **700 nm**

Culture Media:

DMEM/F-12 catalog number: **cytiva SH30023.01**

MEM catalog number: **Gibco 51200-038**

Accutase catalog number: **Innovative cell technologies AT-104**

mTESR+ basal medium catalog number: **Stem Cell Technology 100-0274**
mTESR+ 10x suplement: **Stem Cell Technology 100-0275**
mTESR Plus kit: **Stem Cell Technology 100-0276**

mTESR+ supplemented media
- Add 100ml of mTESR 10x supplement into 400ml of mTESR+ media
- Filter and aliquot 45ml into conical tubes, keep at -20°C.

mFreSR media catalog number: **Stem Cell Technology 05855**

Matrigel preparation

1 Thaw Matrigel stock stored at -80 °C by placing on ice and leaving at 4 °C Overnight

2 Place a 10ml pipette, 1 ml pipete tips and 1.5ml eppendorf tubes to cool before preparing Matrigel aliquots

3 Place Matrigel stock on ice and mix with the previously cooled 10 ml pipette. Make 400 µL aliquots of Matrigel to avoid multiple freeze-thaw cycles using previously cooled tips and tubes.

4 Store 400 µL Matrigel aliquots at -20 °C until use

5 To prepare matrigel working solution thaw 400 µL Matrigel aliquots On ice or at 4 °C

6 Dilute 400 µL Matrigel aliquot in 40 mL of MEM (1:100 dilution)

7 Mix well before use. Store at 4 °C protected from the light

Medium Preparation

8 Prepare mTESR+ media by adding 100 mL of mTESR+ 5x supplement into 400 mL of mTESR+ media and filter it.

9 Add 1 mL of Primocin (50mg/ml) to the mTESR+ media (final concentration 100ug/mL)

Matrigel Coating 5h

10 Before thawing or passaging cells, coat wells with 1 mL of Matrigel per well of 6 well plates or per 35 mm plate

11 Leave coated plates for 01:00:00 to 04:00:00 in the incubator at 37 °C 5h

12 Store diluted matrigel at 4 °C

iPSC thawing 5m

13 Take out 2 mL of mTESR+ per cell line and leave at room temperature to warm up

14 Take out 5 mL of DMEM/F-12 per cell line and leave at room temperature to warm up

15 Label 15 mL conical tubes per line to thaw

16 Take out iPSC vials in dry ice

17 Thaw iPSC vials using a 37 °C water bath until a small piece of ice is left in the vial

18 Using a 1 ml pipette mix the cells gently and transfer to labeled 15 mL conical tubes.

19 Add 5ml of DMEM/F-12 dropwise to the conical tube with the cells

20 Place conical tubes in centrifuge and spin at 300 x g for 00:05:00 at Room temperature 5m

21 Supplement mTESR media with CETP
Add 1 µL of CET per 1 mL of mTESR+ (1:1000 ratio)
Add 1 µL of P per 1 mL of mTESR+ (1:1000 ratio)

22 Aspirate matrigel from coated plates

23 Add 1 mL of mTESR+ containing CETP to each well

24 Take out cells from the centrifuge. Aspirate supernatant from 15 mL conical tube containing cells, avoid disturbing the cell pellet

25 Resuspend pellet with 1 mL of mTESR+ very gently to avoid dissociating iPSCs into single cells. Transfer all the volume into the well of a 6 well plates or per 35 mm plate, label with the line.

26 Gently mix the plate to evenly spread the cells

27 Ensure the presence of cells in each well by viewing the plate under the microscope (should see floating cells)

28 Incubate plate in the incubator at 37 °C

iPSC passaging

10m

29 Take out 3 mL of MEM per well of 6 well plates or per 35 mm plate in a conical tube (1 mL /well to wash old medium and 2 mL /well to wash out acutase) and leave at room temperature to warm up

30 Take out 1 mL of accutase per well of 6 well plate or per 35mm plate in 15 mL conical tube to warm up at RT

31 Take out 3 mL of mTESR+ per well of 6 well plate or 35 mm plate (2 mL /well for plating and 1 mL /well for resuspension) to warm up at RT

32 Aspirate old medium

33 Add 1 mL of MEM per well of 6 well plate or per 35 mm plate, rock the plate to wash

34 Aspirate MEM

35 Add 1 mL of acutase per well of 6 well plate or per 35 mm plate

36 Place plate in the incubator at 37 °C for 00:05:00 5m

37 Get plate out of incubator and tap plate gently to check that cells are detached. Pippete cells once before removing from plate, avoid pippeting too much.

38 Transfer detached cells into a labeled 15 mL conical tube with 2 mL of MEM

39 Place conical tubes in centrifuge and spin at 300 x g for 00:05:00 at Room temperature 5m

40 Supplement mTESR plating media with CETP
Add 1 µL of CET per 1 mL of mTESR+ (1:1000 ratio)
Add 1 µL of P per 1 mL of mTesr+ (1:1000 ratio)

41 Aspirate matrigel from coated plates

42 Add 2 mL of mTESR+ containing CETP to each well

43 Take out cells from the centrifuge. Aspirate supernatant from 15 mL conical tube containing cells, avoid disturbing the cell pellet

44 Resuspend pellet with 1 mL of mTESR+ very gently to avoid dissociating iPSCs into single cells

45 Add 70-100 µL of cell solution per well in 6 well plate or per 35 mm plate (on top of the mTESR+ with CETP)

46 Gently mix the plate to evenly spread the cells

47 Ensure the presence of cells in each well by viewing the plate under the microscope (should see floating cells)

48 Incubate plate in the incubator at 37 °C

iPSC Maintainance

49 After thawing or passaging cells aspirate old medium the next day to wash away the CETP

50 Add 2 mL of fresh mTESR+ to each well of the 6 well plate or per 35mm plate

51 Cell can be fed every other day (or daily depending on cell confluency and growth) until they are ready for splitting again when cells are 70-80% confluent. Cells are usually ready for splitting every 4 days.

Check cells under microscope to estimate confluency

iPSC Freezing

52 Have freezing vials set and labeled with the correct info (iPSC line number, passage number, gene mutation, date, etc).

53 Take out 3 mL of MEM per well of 6 well plates or per 35 mm plate in a conical tube (1 mL /well to wash old medium and 2 mL /well to wash out acutase) and leave at room temperature to warm up

54 Take out 1 mL of accutase per well of 6 well plate or per 35mm plate in 15 mL conical tube to warm up at RT

55 Follow the same protocol shown above for cell passaging for the steps 32-39.

56 Take out cells from the centrifuge. Aspirate supernatant from 15 mL conical tube containing cells, avoid disturbing the cell pellet

57 Resuspend pellet with 1 mL of mFreSR very gently

58 Add 500 µL of cell solution per freezing vial.

59 Place freezing vials in cryogenic freezing container to prevent ice crystals from forming within the cells and store at -80 °C Overnight

60 Transfer vials to a liquid nitrogen tank for storage

Protocol references

Chen, Y., Tristan, C.A., Chen, L., Jovanovic, V.M., Malley, C., Chu, P.H., Ryu, S., Deng, T., Ormanoglu, P., Tao, D., et al. (2021). A versatile polypharmacology platform promotes cytoprotection and viability of human pluripotent and differentiated cells (Springer US).