Nov 13, 2023 Version 1

iPSCs Maintenance and Banking V.1

DOI
https://dx.doi.org/10.17504/protocols.io.ewov1qd5ogr2/v1

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Protocol Citation: Mahmoud ElAchwah, Sol Diaz de Leon, Zhiping Pang 2023. iPSCs Maintenance and Banking. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ewov1qd5ogr2/v1

Protocol status: Working
We use this protocol and it's working

Created: September 15, 2023

Last Modified: November 13, 2023

Protocol Integer ID: 87845

Keywords: pluripotent stem cell, induced pluripotent stem cell, ipscs maintenance, ipsc, cell

Abstract

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

Materials

Matrigel:

Matrigel catalog number: **354234**
Matrigel LoT number: **13823002**
Matrigel stock concentration: **9-11 µg/µL**
Matrigel working concentration: **90-110 µg/mL**
Matrigel Stock aliquot volume: **400 µL**

Culture Antibiotic:

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

CEPT:

Chroman catalog number: **HY-15392**
Chroman working concentration: **50 nM**
Chroman stock concentration: **0.5 mM**

Emricasan catalog number: **S7775**
Emricasan working concentration: **5000 nM**
Emricasan stock concentration: **50 mM**

Polyamine supplement catalog number: **P8483**
Polyamine supplement working concentration: **NA**
Polyamine supplement stock concentration: **NA**

Trans-ISRIB catalog number: **5284**
Trans-IRIB working concentration: **700 nm**
Trans-IRIB stock concentration: **7 mM**

Culture Media:

MEM catalog number: **51200-038**
MEM LoT number: **2451155**

Accutase catalog number: **AT-104**
Acutase LoT number: **2B2527A**

mTesr+ basal medium catalog number: **100-0274**
mTesr+ LoT number: **AH29845535**
mTesr+ 10x suplement: **100-0275**
mTesr Plus kit: **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:

mFreSR media catalog number: **05855**
mFreSR media LoT number: **NA**
mFreSR media stock concentration: **NA**
mFreSR media working concentration: **NA**
mFreSR media Stock aliquot volume: **50 mL**

Diluting Matrigel

1 Thaw Matrigel stock [M] 9-11 µg/µL stored at -80 °C on ice

2 Make 400 µL aliquots of Matrigel to avoid multiple freeze-thaw cycles using previously cooled tips and tubes

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

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

5 Dilute 400 µL Matrigel aliquot in 40 mL of MEM (Working Concentration 90-110 µg/mL)

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

Matrigel Coating

1h

7 Coat wells with 1 mL of Matrigel per well of 6 well plates or per 35 mm plate

8 Leave coated plates for 02:00:00 to 04:00:00 in the incubator at 37 °C

6h

9 Store diluted matrigel at 4 °C

Medium Preparation For Passaging

10 Prepare mTesr+ by adding 100 mL of mTesr+ 10x supplement into 400 mL of mTESR+ media and filter it.

11 Add 1 mL of [M] 50 Molarity (M) Primocin (final concentration 100ug/mL)

12 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

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

14 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

Washing iPSCs

15 Put iPSC plate out of the incubator at Room temperature

16 Aspirate old medium

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

18 Aspirate Wash

Detaching Adherent Cells

4m

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

20 Place plate in the incubator at 37 °C for 00:05:00

5m

Centrifuging Cells

21 Get plate out of incubator and check that cells are detached, avoid pippeting too much.

Add 2 mL of MEM

22 Transfer detached cells with MEM (3 mL total volume) into 15 mL conical tube

23 Place conical tubes in centrifuge and spin at 1000 rpm for 00:05:00 at Room temperature 5m

Preparing Seeding Medium

24 Add 1 µL of CET per 1 mL of mTESR+ (1:1000 ratio)

25 Add 1 µL of P per 1 mL of mTesr+ (1:1000 ratio)

26 Mix mTESR+ with CETP

27 Aspirate matrigel from coated plates

28 Add 2 mL of mTesr+ containing CETP to each well

Collecting Cells

29 Aspirate supernatant from 15 mL conical tube containing cells (should have pellet)

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

31 Add 50 µL of cell solution per well in 6 well plate or per 35 mm plate (on top of the mTESR+ with CETP)

32 Gently mix the plate to evenly spread the cells

Incubation

5m

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

34 Incubate plate in the incubator at 37 °C Overnight

5m

Changing iPSC medium

35 Aspirate old medium the next day to wash the CETP

36 Add 2 mL of fresh mTESR+ to each well of the 6 well plate or per 35mm plate (no wash with MEM first day after splitting)

Wash and iPSC Medium Change

37 Wash CETP from each well/plate with 1 mL of MEM

38 Aspirate wash

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

No need to keep washing plates after the first wash, you can directly change medium

iPSC Maintainance

40 Keep feeding cells everyday until they are ready for splitting again (cells are usually ready for another splitting in 2-3 days, when their confluency reach 70%)

Check cells under microscope to estimate confluency

iPSC Freezing

41 **For freezing purposes**, follow the same protocol shown above for cell maintenance for the following sections: Washing iPSCs, Detaching Afdherent cells, centirfuging cells (steps 10-23)

42 Instead of collecting cells have freezing vials set and barcoded with iPSC line number, passage number, gene mutation and date

43 Resuspend pellet with 1 mL of mFreSR very gently

44 Add 500 µL of cell solution to freezing vials

iPSC Banking

45 Store freezing vials at -80 °C in a cryogenic freezing container to prevent ice crystals from forming within the cells in the freezing vials

46 After 24 hours, move freezing containers with vials from -80 °C to a nitrogen tank