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iNDI Maintenance protocol of iPSCs Version 1

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ABSTRACT

IPSC maintenance protocol

- Matrigel procedure for coating plates
- Vitronectin procedure for coating plates
- Thawing iPSC
- Splitting and Passaging iPSC
- Freezing iPSC

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

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MATERIALS

- Matrigel hESC-qualified (Corning Cat# 354277) **Corning Catalog #354277**
- Vitronectin (VTN-N) Recombinant Human Protein Truncated **Thermo Scientific Catalog # A31804**
- Essential 8™ Medium **Gibco - Thermo Fischer Catalog #A1517001**
- • Chroman I **MedChemExpress Catalog #HY-15392**
- • Phosphate Buffered Saline (1X) without Calcium or Magnesium **Lonza Catalog 17-516F**
- 0.5 mM EDTA **Gibco - Thermo Fischer Catalog # AM9260G**
- Accutase **Gibco - Thermo Fischer Catalog # A1110501**
- Dimethylsulfoxide (DMSO) **Corning Catalog #25-950-CQC**
- Knockout™ Serum Replacement **Gibco - Thermo Fischer Catalog #10828028**
- KnockOut™ DMEM/F-12 **Gibco - Thermo Fischer Catalog #12660012**

Matrigel Coating

1h 30m

1 **Aliquot concentrated Matrigel**:

- Gradually thaw a 5ml bottle of Matrigel on ice in a Styrofoam container
- Pre-chill labeled Eppendorf tubes by placing in a cool rack on ice.
- Before pipetting concentrated Matrigel into pre-chilled tubes, chill a 1 ml pipet tip by pipetting ice-cold KnockOut™ DMEM/F-12 up and down several times, then immediately use the tip to aliquot Matrigel.
- Prepare aliquots of 500 µL of concentrated Matrigel and freeze down at -80 °C

Note

Matrigel concentrated can polymerize rapidly at room temperature, so it's very important when aliquoting or preparing coating solution do on ice.

2 **Coating plates with Matrigel solution:** 1h 30m

- Reconstitute a 500 µL aliquot of Matrigel in 50 mL of cold KnockOut™ DMEM/F-12, pipet 500 µL cold media into the aliquot of Matrigel tube and pipet up and down several times, then transfer what has thawed to the tube containing 50 mL of cold KnockOut™ DMEM/F-12, repeat until the frozen aliquot of Matrigel has been completely transferred to the 50 ml of cold media. Mix inverting several times.

> Note
>
> *Matrigel solution can be store at 4°C until it finishes.*

- Add half of the normal culture volume of Matrigel solution to the culture surface (i.e., 1 ml per well of a 6-well plate).
- Gently rock plate to spread the Matrigel solution evenly across the plate.
- Place in 37 °C incubator for 00:30:00 to 01:00:00 before use it.

> Note
>
> *For KOLF2.1 iPSC you can get better results from* Overnight *coating*

- Aspirate Matrigel and add culture medium (E8).

There is another option for coating plates besides Matrigel. **Vitronectin** is a recombinant human protein that provides a defined surface for feeder-free culture of iPSC. When used with E8 medium, vitronectin has been proven to maintain pluripotency and normal growth characteristics in multiple iPSC lines.

Vitronectin Coating 30m

3 **Aliquot concentrated Vitronectin 0.5 mg/ ml** [M] 0.5 mg/mL

- Thaw a vial of Vitronectin on ice in a Styrofoam container.
- Prepare aliquots of 60 µL of concentrated Vitronectin and freeze down at -80 °C

4 **Coating plates with Vitronectin solution** [M] 5 µg/mL

- Reconstitute a 60 µL aliquot of Vitronectin into a 15 ml conical tube containing 6 mL of PBS, gently resuspend by pipetting up and down, do not vortex.
- Add half of the normal culture volume of Vitronectin solution to the culture surface (i.e. 1 ml per well of a 6-well plate).
- Gently rock plate to spread the Vitronectin solution evenly across the plate.
- Place in 37 °C incubator for Overnight before use it.
- Aspirate Vitronectin and add culture medium (E8).

Thawing iPSC

5m

5 Remove iPSC stock cryovial from liquid nitrogen and thaw in 37 °C bead bath. Thaw quickly by gently swirling until a small piece of frozen material remains. Spray the vial with 70% ethanol before transferring to a biological safety cabinet.

Note

Since DMSO is toxic to cells at room temperature, perform the following steps in a time-efficient manner to obtain optimal cell viability.

6 Gently add the thawed cell suspension dropwise to a conical tube containing 10 mL culture medium or PBS, rinse cryovial with 1ml of medium and add the rinse to the tube, gently mix cells by swirling.

7 Centrifuge tube 00:05:00 at 200 - 300 x g at Room temperature 5m

8 Aspirate the supernatant and gently resuspend cells in culture medium (E8) supplemented with

[M] 50 nanomolar (nM) Chroman I and transfer to Matrigel or Vitronectin-coated plates.

Note

After thawing the cells is recommended to maintain high cell density to maximize cell viability. The table below suggests some number of cells and vessel to use.

Number of cells in cryovial	Vessel
3 x 106 cells	100 mm dish
0.5 x 106 to 1 x 106 cells	a well of 6 well plate
0.2 x 106 to 0.4 x 106	a well of 12 well plate
0.08 x 106 to 0.15 x 106	a well of 24 well plate

9 Gently rock plate to evenly distribute cells.

10 Return plate to 37 °C incubator.

11 Next day replace the media with fresh E8 medium (2 mL /well of a 6-well plate).

12 When the well is 80% confluent pass to expand stock.

Splitting/Passaging iPSC

24m

13 EDTA

13.1 Aspirate culture medium and wash with PBS 1X.

13.2 Aspirate PBS and add half of culture volume (1ml/well of a 6-well plate) of EDTA [M] 0.5 millimolar (mM) in PBS.

> Note
>
> *I have been using* Cell Dissociation Buffer, enzyme-free, PBS **Thermo Fisher Catalog #13151014** as well and it works the same as EDTA.

13.3 Incubate for 00:03:00 at 37 °C or 00:08:00 at Room temperature

11m

> Note
>
> *The time can vary by cell line and density (the optimal density is 70-90%)*

13.4 Aspirate EDTA solution, the cells colonies should remain attached so be careful not to disturb them.

13.5 Add 1 mL of culture medium supplemented with [M] 50 nanomolar (nM) Chroman I to cells to dissociate by pipetting two or three times.

13.6 Typically splitting ratios for 6 well plates are between 1:6 and 1:12, so, add the desire volume of culture medium to the cells and discard any excess of cells or re-plate into a new Matrigel or Vitronectin-coated well.

14 Accutase

14.1 Aspirate culture medium and wash with PBS 1X.

14.2 Aspirate PBS and add half of culture volume of Accutase.

14.3 Transfer to 37 °C incubator for 00:08:00 8m

> Note
>
> *The time can vary by cell line and density (the optimal density is 70-90%) and the goal to use accutase is singularize as single cells.*

14.4 Meanwhile aspirate Matrigel/Vitronectin from plates and add culture medium E8 supplemented with [M] 50 nanomolar (nM) Chroman I.

14.5 When Incubation is ready, tilt the plate and pipet the accutase solution two to three times up and down the culture surface to break the colonies.

14.6 Quench the Accutase adding half of the culture volume of PBS.

14.7 Transfer to a new conical tube and rinse with more PBS the culture surface, combine with the cell solution in the tube.

14.8 Centrifuge 00:05:00 at 200 - 300 x g at Room temperature 5m

14.9 Aspirate supernatant.

> Note
>
> *Remains of Accutase could interfere with the cell viability after re-plating, make sure you aspirate everything.*

14.10 Resuspend the cell pellet in culture medium E8 supplemented with [M] 50 nanomolar (nM) Chroman I.

14.11 Count cells and plate cells the desire amount into a Matrigel-coated plates.

14.12 Gently rock plate to evenly distribute cells.

14.13 Return plate to 37 °C incubator.

14.14 The next day replace the medium with fresh E8 (2ml/well of a 6-well plate)

14.15 When the well is 80% confluent pass to achieve an assay or to expand stock.

Note

KOLF2.1 iPSC does not behave well when is more than 80-90%, most of the cells will die and it will be very difficult to get them back.

Freezing iPSC

15 Prepare freezing medium as combining Knockout™ Serum Replacement with 10% DMSO.

16 Leftovers of EDTA or Accutase dissociation procedure could be cryopreserved by centrifuging as in step 14.8 and resuspending the cell pellet with freezing medium.

17 When freezing cells from a well/plate, prepare the cells as for EDTA split.

18 Aspirate EDTA and gently dissociate cells with freezing medium.

19 Transfer 1 mL of cell suspension to a 1 mL cryovial and freeze in a CoolCell freezing container at -80 °C
Overnight

20 Next day transfer the cryovials to liquid nitrogen for long term storage.