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🌐 iNDI Maintenance protocol of iPSCs Version 1

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We use this protocol and it's working

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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

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 Fisher Scientific 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 Fisher Scientific Catalog # AM9260G**

✂ Accutase **Gibco - Thermo Fisher Scientific Catalog # A1110501**

✂ Dimethylsulfoxide (DMSO) **Corning Catalog #25-950-CQC**

✂ Knockout™ Serum Replacement **Gibco - Thermo Fisher Scientific Catalog #10828028**

✂ KnockOut™ DMEM/F-12 **Gibco - Thermo Fisher Scientific 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 0.5 mg/mL

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

4 Coating plates with Vitronectin solution 5 Mass Percent

- Reconstitute a  60 μ L 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  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 10 ⁶ cells	100 mm dish
	0.5 x 10 ⁶ to 1 x 10 ⁶ cells	a well of 6 well plate
	0.2 x 10 ⁶ to 0.4 x 10 ⁶	a well of 12 well plate
	0.08 x 10 ⁶ to 0.15 x 10 ⁶	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  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 **1M** 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.