Sep 06, 2022

Thawing of hPSCs grown on MEFs

In 1 collection

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1 *Works for me* Share

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ABSTRACT

This protocol describes the standard procedure of thawing human pluripotent stem cells (hPSCs) on inactivated mouse embryonic fibroblasts (MEFs).

General notes

1. Throughout this protocol, the term hPSC is used to collectively refer to both hiPSCs and hESCs. All described procedures have been tested and work equally well for hiPSCs and hESCs.
2. Until otherwise indicated, hPSCs are routinely grown in a humidified cell culture incubator under “low” oxygen conditions. We have successfully maintained hPSCs using either 3% O2 (3% O2, 5% CO2) or 5% O2 (5% O2, 5% CO2) conditions.
3. While freezing hPSCs as single cell solution (using Rock Inhibitor) results in better cell recovery, some laboratories prefer freezing of hPSCs as cell clusters. We have used both approaches and do not observe obvious differences.

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PROTOCOL CITATION

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MATERIALS TEXT

A	B	C
Item	**Vendor**	**Catalog #**
DMEM/F12	Thermo Fisher	11320082
DPBS w/o Calcium and magnesium (DPBS)	Corning	MT21031CV
Fetal Bovine Serum (FBS)	Corning	35-011-CV
Knockout Serum Replacement	Thermo Fisher	10828-028
L-Glutamine	Sigma	G8540
Penicillin & Streptomycin (100X)	Thermo Fisher	15140163
MEM Non-Essential Amino Acids (100X)	Thermo Fisher	11140050
Heat Stable Recombinant Human FGF2	Thermo Fisher	PHG0360
DMSO	Fisher Scientific	BP231-100
BSA	Sigma	A4503
Y-27632	Chemdea	CD0141
2-Mercaptoethanol	Sigma	M3148
0.25% Trypsin with EDTA (Trypsin)	Thermo Fisher	25200114

1 Prepare one 6-well MEFs plate for each vial of frozen hPSCs

2 Place vial of frozen hPSCs in **37 °C** water bath with constant agitation.

3 Pipette thawed cell suspension into 10 ml pre-warmed hPSCs medium.

3.1 hPSCs Medium

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A	B
DMEM/F12	385 ml
Fetal Bovine Serum (FBS)	75 ml
Knockout Serum Replacement	25 ml
L-Glutamine (100X)	5 ml
Penicillin & Streptomycin (100X)	5 ml
MEM Non-Essential Amino Acids (100X)	5 ml
2-Mercaptoethanol (10,000X)	50 µl
Heat Stable Recombinant Human FGF2 (25µg/ml)*	80 µl

*While we prefer Heat Stable Recombinant Human FGF2, we also have used regular FGF2.
Final volume: 500ml

L-Glutamine (100X)

L-Glutamine, powder	14.6 g
MilliQ H2O	500 ml

2-Mercaptoethanol (10,000X)

2-Mercaptoethanol	0.78 ml
MilliQ H2O	9.22 ml

Heat Stable Recombinant Human FGF2 (25µg/ml)

A	B
Heat Stable Recombinant Human FGF2	500 µg
0.1% BSA	20 ml

Final volume: 20ml

4 Centrifuge **200-300 x g, 00:05:00** 5m

5 While cells are spinning, aspirate the MEFs medium from the MEFs plates and add 2 ml pre-warmed hPSCs medium + Rock inhibitor to each well.

While it is necessary to use Rock-Inhibitor to thaw hPSCs frozen as single cell solution, it is

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possible to omit Rock-Inhibitor at this stage to thaw hPSC lines frozen as cell aggregates (see general notes).

5.1 hPSCs Medium + Rock inhibitor

A	B
hPSCs medium	500 ml
Y-27632 (1,000X)	500 µl

Final volume: 500ml

Y-27632 (1,000X)

Y-27632	5 mg
DMSO	1.56 ml

6 Aspirate most of the medium on the centrifuged hPSCs, being careful not to disturb the pellet

7 Add 1 ml hPSCs medium with or without Rock inhibitor (dependent on choice made above).

8 Resuspend the cells using a P1000 tip.

9 Pipette the cells onto the 2 ml already on the MEFs.

10 Check the cells under the microscope to get an idea of the resulting cell density.

11 Spread the cells by moving the plate in left-right, then backward-forward motion.

12 Place the plate in the low oxygen incubator

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13 From day 3, change 2-3 ml pre-warmed hPSCs medium for each well daily.

14 When large colonies emerge or hPSCs density reaches 50-70%, passage using collagenase. It usually takes 7-10 days for the thawed cells to grow to this point.

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