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Thawing of feeder-free hPSCs

In 1 collection

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ABSTRACT

This protocol describes the procedure of thawing feeder-free human pluripotent stem cells (hPSCs) using mTeSR-plus or StemFlex

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, feeder-free 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. We have routinely maintained feeder-free cells in either mTeSR-plus or StemFlex. However, these two mediums are not interchangeable. Pick one and stick to it.
4. We have routinely maintained feeder-free hPSC cultures on VTN, Matrigel and Geltrex--coated cell culture plates without observing obvious differences.

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PROTOCOL CITATION

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COLLECTIONS

Feeder-free culturing of hPSCs

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MATERIALS TEXT

Item	Vendor	Catalog #
DMEM/F12	Thermo Fisher	11320082
mTeSR-plus	STEMCELL Technologies	100-0276
StemFlex	Thermo Fisher	A3349401
Penicillin & Streptomycin (100X)	Thermo Fisher	15140163
Vitronectin (VTN-N) Recombinant Human Protein, Truncated	Thermo Fisher	A14700
DMSO	Fisher Scientific	BP231-100
Y-27632	Chemdea	CD0141
Accutase	Thermo Fisher	SCR005
Matrigel	Corning	CV40234
Geltrex	Fisher Scientific	A1413302
ReLeSR	Stem Cell Technologies	05872

Note: This protocol makes reference to protocols in other collections. Please check for any materials found in those protocols, which might not be listed here

1 Prepare one 6-well VTN/Matrigel/Geltrex-coated plate for each vial of frozen hPSCs.

A detailed protocol on "Coating plates" can be found in the "Feeder-free culturing of hPSCs" collection. A link to this collection can be found in the title section of this protocol, located above

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

3 Pipette thawed cell suspension into 10 ml pre-warmed DMEM/F12.

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

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5 While cells are spinning, aspirate the VTN/Matrigel/Geltrex solution from the coated plates and add 2 ml pre-warmed Feeder-free medium + Rock inhibitor to each well.

5.1 Feeder-free Medium (version A)

StemFlex basal medium	450 ml
StemFlex supplement	50 ml

Final volume: 500ml

Feeder-free Medium (version B)

mTeSR-plus basal medium	400 ml
mTeSR-plus supplement	100 ml

Final volume: 500ml

- Feeder-free mediums (version A & B) are not interchangeable. Pick one and stick to it.
- It is possible to include 5 ml Penicillin & Streptomycin (100X) in the feeder-free medium

Y-27632 (1,000X)

Y-27632	5 mg
DMSO	1.56 ml

Feeder-free medium + Rock inhibitor

A	B
Feeder-free medium	50 ml
Y-27632 (1,000X)	50 µl

Final volume: 50ml

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

7 Add 1 ml Feeder-free medium + Rock inhibitor

8 Re-suspend the cells using a P1000 tip.

9 Dispense the cells onto the VTN/Matrigel/Geltrex-coated plate, adding 160 µl per well.

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

13 Change 2 ml pre-warmed Feeder-free medium for each well every other day. When large colonies emerge or hPSCs density reaches 50-80%, passage using Accutase or ReLeSR. It usually takes 5-7 days for the thawed cells to grow to this point.

A detailed protocol on passaging using Accutase or ReLeSR can be found in the "Passaging of hPSCs" protocol, in the "Feeder-free culturing of hPSCs" collection. A link to this collection can be found in the title section of this protocol, located above

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