Sep 06, 2022

Freezing of feeder-free hPSCs

In 1 collection

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dx.doi.org/10.17504/protocols.io.b4mbqu2n

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ABSTRACT

This protocol describes the process of freezing feeder-free human pluripotent stem cells (hPSCs) using Accutase or ReLeSR

Protocol overview:
A. Accutase
B. ReLeSR

General Notes:
1. For this protocol, hPSCs refers collectively to 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

Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner 2022. Freezing of feeder-free hPSCs. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b4mbqu2n

FUNDERS ACKNOWLEDGEMENT

Aligning Science Across Parkinson's
Grant ID: ASAP-000486

COLLECTIONS

Feeder-free culturing of hPSCs

KEYWORDS

ASAPCRN

CREATED

Feb 02, 2022

LAST MODIFIED

Sep 06, 2022

PROTOCOL INTEGER ID

57731

PARENT PROTOCOLS

Part of collection
Feeder-free culturing of hPSCs

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MATERIALS TEXT

Item	Vendor	Catalog #
DMEM/F12	Thermo Fisher	11320082
DPBS w/o Calcium and magnesium (DPBS)	Corning	MT21031CV
mTeSR-plus	STEMCELL Technologies	100-0276
StemFlex	Thermo Fisher	A3349401
FB Essence	Avantor	10803-034
DMSO	Fisher Scientific	BP231-100
Accutase	Thermo Fisher	SCR005
Styrofoam microtube freezer box	Labnet	R8000
Nalgene® Mr. Frosty® Cryo 1°C Freezing Containers	Thermo Fisher	
ReLeSR	Stem Cell Technologies	05872
Cell lifter	Corning	3008

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

A. Accutase 5m

1 hPSCs are ready to be frozen when the culture reaches 50-80% confluency.

2 Before starting:
a. Prepare Freezing Medium I and II and keep on ice.
b. Pre-label appropriate number of cryovials (freeze approx. 2 vials/well of a 6-well plate)

2.1 Freezing Medium I

A	B
Feeder-free medium*	5 ml
FB essence**	5 ml

*The formulation of feeder-free medium can be found in below
**We have used successfully frozen feeder-free hPSCs using KSR instead of FB essence.
Final volume: 10ml

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Freezing Medium II

FB essence*	8 ml
DMSO	2 ml

*We have used successfully frozen feeder-free hPSCs using KSR instead of FB essence. Final volume: 10ml

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) into the feeder-free medium

3 Wash hPSCs with DPBS.

4 Use 1 ml Accutase/well of a 6-well plate.

5 Incubate **00:05:00** **37 °C** 5m

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6 Add 2 ml DMEM/F12 to each well.

7 Collect all cells into 15 ml conical tube.

8 Add 7 ml DMEM/F12.

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

10 Aspirate supernatant

11 Gently re-suspend the pellet in 500 µl Freezing Medium I per vial to be frozen, triturate 5-10 times to achieve single cell suspension using a P1000 tip

12 In each cryovial, add 500 µl pre-chilled Freezing Medium II.

13 Dispense 500 µl cell suspension into each cryovial using P1000, mix

14 Temporarily keep cryovials on ice until all cells dispensed.

15 Place cryovials into Styrofoam microtube freezer box or pre-cooled (4°C on ice) NALGENE™ Cryo 1°C Freezing Container filled with 250 ml of Isopropanol.

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16 Freeze at **-80 °C** **Overnight**

17 For long term storage, store cryovials in liquid nitrogen (-196ºC).

B. ReLeSR 12m

18 hPSCs are ready to be frozen when the culture reaches 50-80% confluency.

19 Before starting:
a. Prepare Freezing Medium I and II and keep on ice.
b. Pre-label appropriate number of cryovials (freeze approx. 2 vials/well of a 6-well plate)

19.1 **Freezing Medium I**

A	B
Feeder-free medium	5 ml
FB essence*	5 ml

*We have used successfully frozen feeder-free hPSCs using KSR instead of FB essence .
Final volume: 10ml

Freezing Medium II

FB essence*	8 ml
DMSO	2 ml

*We have used successfully frozen feeder-free hPSCs using KSR instead of FB essence .
Final volume: 10ml

20 Wash the plates twice with DPBS

21 Add 1 ml/well of ReLeSR to a 6-well plate and incubate for **00:01:00** 1m
Room temperature

22 Remove the solution and let it sit at **Room temperature** for **00:02:00** 2m

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23 Add 2 ml of Feeder-free medium/well.

24 Gently scrape the cells from the bottom of the well by using a Corning cell lifter.

25 Collect the cells in a 15 ml conical tube and add 3 ml of Feeder-free medium.

26 Gently mix by inversion and gravity precipitate for **00:05:00** 5m

27 Remove the supernatant down to 1 ml and add 5 ml of Feeder-free medium.

28 Pellet the cells at **150 x g, Room temperature, 00:04:00** and aspirate the supernatant. 4m

29 Gently re-suspend the pellet in 500 µl Freezing Solution I per vial to be frozen.

30 Carefully add 500 µl Freezing Medium II per vial to be frozen.

31 Dispense 1ml aliquots in pre-labeled cryovials and keep on ice.

32 Place cryovials into Styrofoam microtube freezer box or pre-cooled (4°C on ice) NALGENE™ Cryo 1°C Freezing Container filled with 250 ml of Isopropanol.

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33 Freeze **Overnight** **-80 °C**

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