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Passaging of feeder-free hPSCs

In 1 collection

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ABSTRACT

This protocol describes the process of passaging human pluripotent stem cells (hPSCs) for feeder-free culturing of hPSCs using Accutase or ReLeSF

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.
5. We have routinely passaged feeder-free hPSCs using either Accutase (as single cell suspension) or ReLeSR (as cell aggregates) without observing obvious differences.

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COLLECTIONS

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
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
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

BEFORE STARTING

Prior to passaging, prepare VTN/Matrigel/Geltrex-coated plates. A detailed protocol for this procedure can be found in the protocol "Coating plates," which is located 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

A. Accutase 10m

1 Wash hPSCs with DPBS

2 Add 1 ml Accutase/well to the 6-well plate.

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3 Incubate **00:05:00** **37 °C** 5m

4 Add 2 ml DMEM/F12 to each well.

5 Collect all cells into 15 ml conical tube.

6 Add 7 ml DMEM/F12.

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

8 Aspirate supernatant

9 Re-suspend cell pellet in 1 ml Feeder-Free Medium + Rock inhibitor. Use a P1000 tip and triturate 5-10 times to achieve single-cell suspension

9.1 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

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- 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

10 Aspirate VTN/Matrigel/Geltrex solution from the coated plate, add 2 ml Feeder-Free Medium + Rock inhibitor to each well.

11 Dispense the proper amount of cells to VTN/Matrigel/Geltrex-coated plates. The splitting ratio differs between cell lines but is usually within a range of 1:6 to 1:30.

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

13 Place the plate in the low oxygen incubator

14 Change 2 ml pre-warmed Feeder-free medium for each well every other day.

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15 When hPSCs density reaches 50-80% confluency, passage again or freeze. It usually takes 5-7 days.

A detailed description for freezing can be found in the "Freezing of feeder-free hPSCs" protocol within the "Feeder-free culturing of hPSCs" collection. A link to this collection can be found in the title section of this protocol, located above

B. ReLeSR 13m

16 Wash the feeder-free hPSCs twice with DPBS

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

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

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

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

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

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

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

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

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25 Remove the supernatant down to 1 ml.

26 Dilute the cells with the desired volume and carefully plate cell aggregates on VTN/Matrigel/Geltrex-coated plates in Feeder-free medium. The splitting ratio differs between cell lines but is usually within a range of 1:6 to 1:30.

27 Spread the cells by moving the plate in left-right, then back-fourth motion.

28 Place the plate in the low oxygen incubator.

29 Change 2 ml pre-warmed Feeder-free medium for each well every other day or if necessary earlier.

30 When hPSCs density reaches 50-80% confluency, passage again or freeze. It usually takes 5-7 days.

A detailed protocol for freezing 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.

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