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Feeder-free culturing of hPSCs

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ABSTRACT

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

Collection overview

Coating plates
- A. VTN
- B. Matrigel
- C. Geltrex

Adapting hPSCs cultured on MEFs to feeder-free system

Thawing of feeder-free hPSCs

Passaging of feeder-free hPSCs
- A. Accutase
- B. ReLeSR

Freezing of feeder-free hPSCs
- A. Accutase
- B. ReLeSR

General Notes

1. Throughout these protocols, 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 media 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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COLLECTION CITATION

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KEYWORDS

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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
Vitronectin (VTN-N) Recombinant Human Protein, Truncated	Thermo Fisher	A14700
DMSO	Fisher Scientific	BP231-100
Y-27632	Chemdea	CD0141
Accutase	Thermo Fisher	SCR005
Collagenase type IV	Thermo Fisher	17104019
Styrofoam microtube freezer box	Labnet	R8000
Nalgene® Mr. Frosty® Cryo 1°C Freezing Containers	Thermo Fisher	
Matrigel	Corning	CV40234
Geltrex	Fisher Scientific	A1413302
ReLeSR	Stem Cell Technologies	05872
Cell lifter	Corning	3008

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FILES

Coating plates
Version 1
by Devin E Snyder

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