# Adapting hPSCs cultured on MEFs to feeder-free system

In 1 collection

Sep 06, 2022

Hanqin Li[1], Oriol Busquets[2], Steven Poser[2], Dirk Hockemeyer[1], Frank Soldner[2]

[1]University of California, Berkeley; [2]Albert Einstein College of Medicine



dx.doi.org/10.17504/protocols.io.b4k5quy6

Devin E Snyder

ABSTRACT

This protocol describes the procedure of adapting human pluripotent stem cells (hPSCs) to feeder-free culturing conditions 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.

DOI

dx.doi.org/10.17504/protocols.io.b4k5quy6

PROTOCOL CITATION

Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner 2022. Adapting hPSCs cultured on MEFs to feeder-free system. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b4k5quy6

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

57725

PARENT PROTOCOLS

Part of collection
Feeder-free culturing of hPSCs

MATERIALS TEXT



**Citation:** Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner Adapting hPSCs cultured on MEFs to feeder-free system https://dx.doi.org/10.17504/protocols.io.b4k5quy6

| Item | Vendor | Catalog # |
|---|---|---|
| DMEM/F12 | Thermo Fisher | 11320082 |
| Knockout Serum Replacement (KSR) | Thermo Fisher | 10828-028 |
| L-Glutamine | Sigma | G8540 |
| Penicillin & Streptomycin (100x) | Thermo Fisher | 15140163 |
| MEM Non-Essential Amino Acids (100X) | Thermo Fisher | 11140050 |
| 2-Mercaptoethanol | Sigma | M3148 |
| Heat Stable Recombinant Human FGF2 | Thermo Fisher | PHG0360 |
| DPBS w/o Calcium and magnesium (DPBS) | Corning | MT21031CV |
| mTeSR-plus | STEMCELL Technologies | 100-0276 |
| StemFlex | Thermo Fisher | A3349401 |
| FBS | Gibco | 10437028 |
| Vitronectin (VTN-N) Recombinant Human Protein, Truncated | Thermo Fisher | A14700 |
| DMSO | Fisher Scientific | BP231-100 |
| Y-27632 | Chemdea | CD0141 |
| Collagenase type IV | Thermo Fisher | 17104019 |
| Matrigel | Corning | CV40234 |
| Geltrex | Fisher Scientific | A1413302 |

**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 When MEFs-cultured hPSCs reach 50% confluency, change medium to hPSCs medium + Rock inhibitor, preparing for the feeder-free adaptation on the next day.

## 1.1 hPSCs medium



**Citation:** Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner Adapting hPSCs cultured on MEFs to feeder-free system https://dx.doi.org/10.17504/protocols.io.b4k5quy6

| 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 (25ug/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

### Y-27632 (1,000X)

| | |
|---|---|
| Y-27632 | 5 mg |
| DMSO | 1.56 ml |

### hPSCs medium + Rock Inhibitor

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

Final volume: 500ml



**Citation:** Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner Adapting hPSCs cultured on MEFs to feeder-free system https://dx.doi.org/10.17504/protocols.io.b4k5quy6

2 Coat three wells of a 6-well plate with either VTN/Matrigel/Geltrex for each cell line.

For a detailed protocol, refer to "Coating plates," which can be found in the protocol collection "Feeder-free culturing of hPSCs." This collection can be accessed using the collection link found in the title section of this protocol, located above

3 Wash one well of MEF-cultured hPSCs with DPBS

4 Add 1 ml Collagenase solution to this well.

### 4.1 Collagenase solution

| A | B |
|---|---|
| Collagenase type IV | 10 mg |
| KSR medium | 10 ml |

Final volume: 10ml

**KSR medium**

| A | B |
|---|---|
| DMEM/F12 | 385 ml |
| Knockout Serum Replacement | 100 ml |
| L-Glutamine (200 mM) | 5 ml |
| Penicillin & Streptomycin (100X) | 5 ml |
| MEM Non-Essential Amino Acids (100X) | 5 ml |

Final volume: 500ml

5 Incubate **00:45:00** **37 °C** . Watch for edge curling of the colonies as this indicates that collagenase incubation is complete. 45m

6 Add 2 ml DMEM/F12



**Citation:** Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner Adapting hPSCs cultured on MEFs to feeder-free system https://dx.doi.org/10.17504/protocols.io.b4k5quy6

7 Pipette repeatedly with 5 ml pipette to lift colonies, careful not to carry over too many MEFs.

8 Collect into 15 ml conical tube.

9 Add 7 ml DMEM/F12.

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

11 Aspirate supernatant

12 Resuspend cell pellet in 1 ml pre-warmed Accutase

13 Incubate **00:05:00** **37 °C** 5m

14 Add 9 ml DMEM/F12, invert to mix

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

16 Aspirate supernatant



**Citation:** Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner Adapting hPSCs cultured on MEFs to feeder-free system https://dx.doi.org/10.17504/protocols.io.b4k5quy6

17 Re-suspend cell pellet in 1 ml Feeder-Free Medium + Rock inhibitor, triturate 5-10 times to achieve single cell suspension using a P1000 tip

17.1 **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) into the feeder-free medium

**Feeder-free medium + Rock Inhibitor**

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

Final volume: 50ml

18 Aspirate VTN/Matrigel/Geltrex solution from the coated plate, add 2 ml Feeder-free medium + Rock inhibitor to each well.

19 Dispense 20 µl, 60 µl, 200 µl cell suspension respectively into the three VTN/Matrigel/Geltrex-coated wells.



**Citation:** Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner Adapting hPSCs cultured on MEFs to feeder-free system https://dx.doi.org/10.17504/protocols.io.b4k5quy6

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

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

22 Place the plate in a low oxygen incubator

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

24 When large colonies emerge or hPSCs density reaches 50-80%, passage the well showing the best hPSCs morphology using Accutase or ReLeSR. It usually takes 5-7 days.

A detailed protocol on "Passaging of feeder-free hPSCs" can be found in the collection "Feeder-free culturing of hPSCs." This collection can be found using the collection link in the title section of this protocol, located above

25 It usually takes 2 passages for hPSCs to fully adapt to feeder-free culture. Differentiation and changes on growth speed are normal during the adaptation.



**Citation:** Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner Adapting hPSCs cultured on MEFs to feeder-free system https://dx.doi.org/10.17504/protocols.io.b4k5quy6