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# Passaging of hPSCs grown on MEFs

In 1 collection

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ABSTRACT

This protocol describes the standard procedure of using collagenase to passage human pluripotenct stem cells (hPSCs) on inactivated mouse embryonic fibroblasts (MEFs).

## 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, 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. While bulk/collagenase passaging is used for routine maintenance of hPSC cultures, manual/microdissection passaging is used to enrich for undifferentiated hPSC colonies (“clean-up” of culture based on undifferentiated hPSC colony morphology) or to expand (“pick”) individual colonies (e.g.,for clonal expansion of individual targeted cells in the process of establishing genome edited cell lines). Manual passaging/ microdissection requires (*i*) the identification and discrimination of undifferentiated and differentiated hPSC colonies and (*ii*) the capacity to excise the undifferentiated cells and transfer them to a new plate and can be performed using various approaches as established in many hPSC laboratories.

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PROTOCOL CITATION

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COLLECTIONS

**Thawing, Passaging and Freezing of hPSCs on MEFs**

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MATERIALS TEXT

| A | B | C |
|---|---|---|
| **Item** | **Vendor** | **Catalog #** |
| DMEM/F12 | Thermo Fisher | 11320082 |
| DPBS w/o Calcium and magnesium (DPBS) | Corning | MT21031CV |
| Fetal Bovine Serum (FBS) | Corning | 35-011-CV |
| Knockout Serum Replacement | Thermo Fisher | 10828-028 |
| Newborn Calf Serum | Sigma | N4762 |
| L-Glutamine | Sigma | G8540 |
| Penicillin & Streptomycin (100X) | Thermo Fisher | 15140163 |
| MEM Non-Essential Amino Acids (100X) | Thermo Fisher | 11140050 |
| Heat Stable Recombinant Human FGF2 | Thermo Fisher | PHG0360 |
| Collagenase type IV | Thermo Fisher | 17104019 |
| BSA | Sigma | A4503 |
| 2-Mercaptoethanol | Sigma | M3148 |
| 0.25% Trypsin with EDTA (Trypsin) | Thermo Fisher | 25200114 |

1 Check MEFs plates for suitability to receive passaged hPSCs prior to beginning. Refer to the "Maintenance and inactivation of mouse embryonic fibroblasts (MEFs) as feeder cells for human pluripotent stem cell culture" collection; dx.doi.org/10.17504/protocols.io.b4pbqvin

2 Wash hPSCs with DPBS

3 Use 1 ml Collagenase solution/well of a 6-well plate.

## 3.1 Collagenase solution



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

Final volume: 10ml

### KSR medium

| | |
|---|---|
| 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

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

5 Add 2 ml wash medium to each well.

### 5.1 Wash medium

| | |
|---|---|
| DMEM/F12 | 470 ml |
| Newborn Calf Serum | 25 ml |
| Penicillin & Streptomycin (100X) | 5 ml |

Final volume: 500ml

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

7 Collect into 15 ml conical tube.

8 Add 4-5 ml Wash Medium.

9 Gravity precipitate cells 5- 10 min.



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10 Aspirate Wash Medium, leave 0.5 ml. Don’t disturb the colonies that are loosely pelleted.

11 Repeat steps 8-10 once

12 Add 5 ml hPSCs medium

### 12.1 hPSCs Medium

| 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)



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| A | B |
|---|---|
| Heat Stable Recombinant Human FGF2 | 500 µg |
| 0.1% BSA | 20 ml |

Final volume: 20ml

13 Repeat steps 9-10 once

14 Add 3 ml hPSCs medium

15 Use a 10 ml strip pipette and triturate the colonies 5-10 times against the bottom of the tube to break up cell clusters

a. The objective is to reduce cluster size, not to completely dissociate to single cells.
b. Avoid introducing air bubbles.

16 In preparation for the hPSCs, plate 1 ml hPSCs medium onto each MEFs well.

17 Add more hPSCs medium to the triturated colonies suspension to a proper dilution so that each MEFs wells will receive 2 ml. Splitting ratio varies between different cell lines and different operators. It's usually within a range of 1:6 to 1:20.

18 Mix well, distribute 2 ml to each MEFs wells.

a. Use of 5 ml pipette to mix cell suspension is preferred as it has the widest bore size, but 10 ml can be used if the volume of cells to plate is large.
b. When mixing, expel and take up the entire volume of medium and cells 2 - 3 times, then take 4 ml of cell suspension to plate two wells at a time.
c. Repeat until all cells are plated.



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19 Place the plate in the low oxygen incubator

a. Do not stack the plates more than two high, preferably leave them as single level.
b. Spread the cells by moving the plate in left-right, then backward-forward motion.
c. Repeat cycle 2 - 3 times.
d. Give the plate a final jerking motion in the backward-forward direction as the last spreading motion.
e. Close the door of the incubator gently as to not disturb the position of the cell clusters.

20 From day 3, change 2-3 ml pre-warmed hPSCs medium for each well daily.

21 When hPSC density reaches 50-70% confluency, passage again. We usually adjust the splitting ratio to passage every 7 days.



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