Sep 06, 2022

Mitomycin C inactivation of mouse embryonic fibroblasts (MEFs) for hPSC cultures

In 1 collection

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ABSTRACT

This protocol describes the process of using Mitomycin C to inactivate mouse embryonic fibroblasts (MEFs), which can then be used as feeder cells for human pluripotent stem cell (hPSC) culture.

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. MEFs were obtained as described in Manipulating the Mouse Embryo: A Laboratory Manual, Third Edition (ISBN: 0879695919)

Andras Nagy, Marina Gertsenstein, Kristina Vintersten, & Richard Behringer. Manipulating the Mouse Embryo: A Laboratory Manual, 3rd ed.. Cold Spring Harbor Laboratory Press.

DOI

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PROTOCOL CITATION

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COLLECTIONS

Maintenance and inactivation of mouse embryonic fibroblasts (MEFs) as feeder cells for human pluripotent stem cell culture

KEYWORDS

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PARENT PROTOCOLS

Part of collection
Maintenance and inactivation of mouse embryonic fibroblasts (MEFs) as feeder cells for human pluripotent stem cell culture

MATERIALS TEXT

Item	Vendor	Catalog #
DMEM	Corning	10-013-CV
FB Essence	Avantor	10803-034
FBS	Gibco	10437028
200mM L-Glutamine	Sigma	G8540
Penicillin & Streptomycin	Gibco	15140-122
MEM Non-Essential Amino Acids	Gibco	11140-050
Gelatin powder	Sigma	G2625
0.25% Trypsin/EDTA (Trypsin)	Gibco	25200-056
DPBS w/o Ca & Mg (DPBS)	Corning	MT21031CV
50ml centrifuge tubes	Corning	1495949A
10cm petri dish	Fisher	08757100D
10ml serological pipet	Corning	7200574
15cm tissue culture dish	Corning	0877224

1 Grow MEFs to 90-95% confluency in 15-cm cell culture dish

2 Aspirate the medium and add 15 ml of Mitomycin C solution to cover the surface

3 Incubate in Mitomycin C for **02:30:00** **37 °C**

2h 30m

4 Aspirate Mitomycin C solution from the plates and wash 4 times with DPBS (with Ca/Mg) and final wash with DPBS (w/o Ca/Mg).

5 Add Trypsin and incubate in 37°C; 5% CO2 for **00:05:00**

5m

6 Add MEF medium to neutralize the Trypsin and collect the solution into a conical tube.

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6.1 MEF medium

A	B
DMEM	435 ml
FB Essence/FBS*	75 ml
200mM L-Glutamine	5 ml
Penicillin & Streptomycin (100x)	5 ml
MEM Non-Essential Amino Acids	5 ml

*We have successfully used either FB Essence or FBS and have not observed an obvious difference. Final volume: 500ml

7 Centrifuge the cell suspension at **250 x g, Room temperature, 00:10:00** 10m

8 Discard supernatant and re-suspend the cells in fresh MEF medium

9 Count cells using trypan blue solution

10 Mitomycin C treated cells can be either freshly plated as feeder cells for hPCS cultures or frozen as Mitomycin C inactivated stocks at $10x10^6$ cells/vial.

A protocol on freezing MEFs can be found in the collection "Maintenance and inactivation of mouse embryonic fibroblasts (MEFs) as feeder cells for human pluripotent stem cell culture." A link to this collection can be found in the title section of this protocol, located above

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