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Passaging and Seeding Mouse Embryonic Fibroblasts (MEFs) for Experiments

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol details the passaging and seeding mouse embryonic fibroblasts (MEFs) for experiments.

Attachments

DOCX

1003-2590.docx

18KB

Passaging and Seeding Mouse Embryonic Fibroblasts (MEFs) for Experiments

15m

1 Passage cells when confluency exceeds 60%.

2 Remove media.

3 Wash cells with 5 mL prewarmed PBS without Calcium and magnesium.

4 Aspirate and discard PBS.

5 Add 3 mL - 5 mL Room temperature TrypLE Express (enough to ensure complete coverage of cells).

6 Incubate cells at 37 °C until they have detached.

6.1 Check at 00:03:00 - 00:05:00 intervals under an inverted microscope, gently tap flask to dislodge cells if needed.

8m

7 Add 5 mL - 10 mL (or at least 1:1 ratio of media : TrypLE) of pre-warmed complete media to flask.

7.1 Pipette up and down to dislodge cells as needed.

8 Transfer cell suspension to conical tube.

9 Spin down at 1300 rpm - 1800 rpm (300 x g) for 3 mins - 00:05:00 .

5m

9.1 During this time label one microcentrifuge tube for each cell line being used and add 20 µL of Trypan blue.

10 Check for cell pellet then aspirate and discard supernatant.

10.1 Avoid disturbing the cell pellet.

11 Resuspend cells in appropriate volume of prewarmed complete media.

12 Take 20 µL of resuspended cell mix (gently pipetting up and down 10 times) and add to 20 µL of trypan blue.

12.1 Pipette up and down 10 times to mix cells with trypan blue.

13 Count the cells using a Countess 3 (Thermofisher Scientific).

13.1 Clean the glass hemocytometer slide and add 10 µL of cell / trypan blue mix to chamber A and B.

13.2 Avoid bubbles and dirt.

14 Perform cells count ensuring working in same units.

Note

e.g. $x10^5$ cells per mL or $x10^6$ cells per mL.

A=_____ B=_____ Average A & B =_____

15 Once counts are performed mix cell suspension again as cells will have settled to the bottom of the tube by the time counts are done.
Add _____ µL/mL of to _____ µL/mL of media

16 Seed cells in appropriate volumes.

17 Record the following:
- Cell type, strain and genotype*
- Number of wells
- Density per well*
- Passage*
- Time and Date of Seeding*
- Temperature(if not at standard room temp)

18 If left over cell are needed, place in an appropriate volume of media and transfer to a fresh flask for future experiments

19 Incubate plate and flasks Overnight (O/N) in 37 °C incubator. 5m