Harvesting MEFs

This is a protocol for isolating mouse embryonic fibroblasts.

Keywords: MEFs mESCs

Expand

Reagents, supplies, and equipment:

- PBS
- trypsin/EDTA
- Supplemented feeder media (DMEM + 10% FBS+ L-glutamine + pen/strep)
- 50 ml conical tubes
- 1 X feeder freezing media
- 1 ml NUNC cryopreservation vials
- Nalgene isopropanol freezing container(s)

Procedure: (starting with a 150 mm dish of confluent cells)

1. Aspirate media, rinse, and aspirate twice with 25 ml PBS.
2. Add 5 ml trypsin/EDTA per dish, roll to cover and place @ 37°C for 5 min.
3. Add 5 ml feeder media; swirl to mix; yielding 10ml/dish. With 25 ml pipette, pipette vigorously several times to break up clumps and rinse bottom of dish to capture loosely adhering cells.
4. Transfer cells from 3 dishes to 50 ml conical tubes and centrifuge @ 3000 rpm for 5 min.
5. Aspirate supernatant, re-suspend pellet in 5 ml feeder media.
6. Pool volumes from all conical tubes into a single 50 ml conical tube, increase volume to 50 ml, seal cap, and invert to mix.
7. Take 1 ml of cell suspension, dilute 1:5 and count cells. To determine # of vials to stock, divide total cell # by 5.0 x 10^6.
8. Take remaining 49 ml ON ICE to be irradiated @ 1,000 rads for 10 min.
   Certification is required by the Department of Environmental Health and Safety in order to operate the irradiator.
9. After irradiation, label Nunc cryovials appropriately with feeder type and batch #. The irradiated feeders are @ p5.
10. Centrifuge conical tube @ 3000 rpm for 5 min, and aspirate supernatant.
11. Add X mls 1x feeder freezing media (X= # of vials to be stocked) and re-suspend cells thoroughly.
12. Aliquot 1 ml into each vial and close cap tightly.
13. Place vials in Nalgene isopropanol freezing chamber (or Styrofoam box) and put in -70°C freezer O/N.
14. Move to liquid nitrogen storage and add to liquid nitrogen database.

Source: ES Cell Core Lab
Revised: 11/24/03 by Jill Lindner