Feeder Preparation from Mouse Embryonic Fibroblasts

updated Apr 11, '08 (SJZ)

NOTE: flasks need not be gelatinized for use

confluent cells usually show swirling patterns or whorls

allow cells to grow until highly confluent before splitting, failure may result in little growth upon splitting

Day 1 Seed Culture

1.thaw 1vial MEFs (5-6x10^6cells from Chemicon/ Millipore: PMEF-HL cells)
2.transfer cells to 15ml conical tube and add 10ml MEF media
3.pellet cells: 1000rpm 5min RT
4.resuspend cells in 1ml MEF media
5.transfer cells to two T75 flasks and add MEF media to 25ml
6.allow cells to grow to confluency

Day 3-5 Spit Cells

1.aspirate media
2.add 3ml trypsin (diluted 1:4 in PBS) and incubate flask in 37°C incubator until detached, ~3-10 minutes
3.quench by adding 7ml MEF media
4.resuspend cells by pipetting 15x
5.split suspension between 4 T175 flasks
6.add MEF media to 50ml
7.allow cells to grow to confluency

Day 5-7 Split Cells

1.aspirate media and add 5ml trypsin (diluted) to each T175, incubate 5min @ 37°C
2.quench with 15ml MEF media and resuspend cells
3.split suspension between 10 T175 flasks
4.allow cells to grow to confluency

Day 8-10 Harvesting, Irradiation and Freezing for Use

1.harvest cells off flasks as done previously
2.quench trypsin activity with 12ml MEF media
3.pool cell suspension to 50ml conical tubes
4.pellet cells: 1000rpm 5min RT
5.resuspend each pellet in 5ml MEF media
6.irradiate cells in Cesium Irradiator. Total dose of 26grays (1gray = 100rads) = 2600 RADS (as of 1/11/07 in Karp, use 3.56min)

irradiator code: 4 + 5; 3; clockwise

7.count a dilution of cells using hemocytometer

total cell count = cell count*10,000*dilution factor*cell volume

8.freeze cells down @ 3x10^6 cells/vial, keep on ice until transferred to -80

MEF MEDIA

450mL Commerical Media (Gibco DMEM low glucose Cat#11885)
50mL serum
5mL penstrep

1X FREEZING MEDIA
35mL MEF media
10mL serum
5mL DMSO