Information for p0 MEFs (Mouse Embryonic Fibroblasts) – Product code: MEF/001

You are provided with the following:

- 2ml cryovial of frozen p0 MEF cells – used to produce frozen banks of inactivated feeder cells.

MEF Feeder Cells			
Species: **Mouse**	Strain: **MF-1 or CF-1**	Passage Number: **Use from p1 - p5**	
Growth medium base solution: DMEM		Invitrogen	41965-039
Supplements	**Final concentration**	**Supplier**	**Catalogue number**
FBS Glutamine	10% 2mM	---------- Invitrogen	---------- 21051-016

Thawing of p0 MEFs & Feeder Cells

1. Remove vial from liquid nitrogen and thaw rapidly at 37°C.
2. Once thawed, transfer contents of vial to a 15 ml centrifuge tube. Slowly add 5 - 10 ml growth medium.
3. Centrifuge at 200 x g for 5 minutes.
4. Remove supernatant and then gently resuspend cell pellet in 10ml growth medium.
5. Transfer to a 175cm^2 flask and add a further 40 - 50ml growth medium.
6. Incubate flasks at 37°C, 5% CO_2. Check cultures the following day.

Note: *Cells can be subcultured at a ratio of 1:3 - 1:4 for up to 5 passages and/or can be inactivated at each passage for use as feeders for hES cells*.

Subculture of MEF Cells

1. Remove medium from flask and wash cells with 15ml PBS.
2. Add 15ml Trypsin/EDTA per 175cm^2 flask. Wash over cells for 10-20 seconds and then discard solution.
3. Incubate at 37°C for 5 minutes. Tap flask to aid detachment of the MEFs.
4. Gently resuspend cells in 10ml growth medium. Add further growth medium to 30ml or 40 ml (for a 1:3 or 1:4 split ratio) and transfer 10ml to each new flask.
5. Add a further 40-50ml growth medium per flask and then transfer cultures to incubator.
6. Incubate at 37°C, 5% CO2 until cells are ready to subculture or inactivate.

Preparation of Inactivated Feeder Cells

1. Prepare mitomycin-C medium (10µg/ml mitomycin-C in growth medium).

2. Remove growth medium from flask of MEFs and replace with enough mitomycin-C medium to cover the cells. Incubate flasks at 37°C, 5% CO_2 for 2-3 hours.

3. Whilst the MEFs are incubating, pre-treat stem cell culture plates/flasks with 0.1% gelatin for at least 1 hour.

4. After incubation, aspirate mitomycin-C medium from cells and wash with PBS. Discard solution.

5. Add 15ml Trypsin/EDTA per 175cm^2 flask. Wash over cells for 10-20 seconds and then discard solution.

6. Incubate at 37°C for 5 minutes. Tap flask to aid detachment of the MEFs.

7. Resuspend cells in 10ml growth medium. Transfer suspension to a 15 ml centrifuge tube.

8. Centrifuge at 200 x g for 5 minutes.

9. Aspirate supernatant and resuspend pellet in 10 ml growth medium.

10. Remove gelatin solution from stem cell culture dishes.

11. Count MEFs and seed at 1.5-2.0x10^4 per cm^2 culture surface.

12. The MEFs can be used after about 5 - 6 hours, but are best left to settle overnight. Use within five days. Alternatively, inactivated cells can be frozen for future use.
    NOTE: Up to 30% of cells may be lost during the freeze/thaw process, so a higher number of thawed inactivated cells should be plated, compared to freshly inactivated.

Cryopreservation of Feeder Cells

1. Remove growth medium from flask and wash cells with 15ml PBS.

2. Add 15ml Trypsin/EDTA per 175cm^2 flask. Wash over cells for 10-20 seconds and then discard solution.

3. Incubate at 37°C for 5 minutes. Tap flask to aid detachment of the MEFs.

4. Resuspend cells in 10ml growth medium. Transfer suspension to a 15 ml centrifuge tube.

5. Centrifuge at 200 x g for 5 minutes.

6. Aspirate supernatant and resuspend cell pellet in cold freezing medium (FBS + 10% DMSO). Allow 2-3 vials per 175cm^2 flask used and 1ml freezing medium per vial produced.

7. Label 2ml vials with cell name, passage level and date frozen.

8. Place vials into a freezing container (e.g. “Mr Frosty”, NUNC) and transfer to -70°C freezer.

9. The next day, transfer vials from -70°C to liquid nitrogen for long-term storage.