

# Mouse Embryonic Feeder Cell Protocol: Subculturing MEFs

*Adapted from Wesselschmidt, R. L. Primogenix, Inc.*

*For research use only*

## Background

Mouse embryonic fibroblasts (MEFs) have been used as feeder cell layers for the culture of embryonic stem cells (ESCs) since the first mouse ESCs were derived in 1981. MEFs continue to be the most commonly used feeder cell type for the culture and maintenance of mouse and human derived ESC lines. MEFs provide a complex, but unknown mixture of nutrients and substrata for the long term growth and proliferation of undifferentiated pluripotent ESCs.

## Required Materials

- Vial of cryopreserved MEFs
- MEF media
- Tissue culture flask (can vary in size depending on seeding density and amount of cells to be thawed)
- Sterile 15 mL centrifuge tube
- General Cell Culture supplies

## Media Preparation

### MEF Media

Thermo Scientific HyClone Product	Volume (500 mL final)	Catalog Number
AdvanceSTEM™ DMEM4SC	440 mL	SH30824.01
ES Screened FBS	50 mL (10%)*	SH30070.03E
AdvanceSTEM ES Qualified L-glutamine 200mM	5.0 mL	SH30852.01
AdvanceSTEM ES Qualified (NEAA) 100X	5.0 mL	SH30853.01
Penicillin / Streptomycin Solution (optional)	5.0 mL	SV30010
Aseptically combine medium, FBS and supplements, then mix by gently inverting a closed container. Store at 4°C. Unused medium should be discarded after six weeks.		
* We also have had good success with 15% FBS		

## General Considerations

MEFs require careful culture and maintenance. Keeping MEFs in a healthy proliferating state producing all of the matrix and growth factor support for ESCs is an important goal. Since MEFs are primary cells, they have a limited lifespan in culture. If they begin to elongate and doubling time increases significantly, they are beyond their useful state. They need to be carefully monitored to avoid over growing the culture which results in early senescence.

The following criteria are recommended when sourcing MEFs. They should be:

- Isolated from 12.5 - 13.5 day mouse embryos
- Mycoplasma free
- Mouse Antibody Production (MAP) tested

### *Subculturing MEFs*

We recommend following the instructions supplied by the provider of the MEFs. Alternatively, our standard protocol is listed below. In all instances, follow proper aseptic technique and work under appropriate tissue culture hood where applicable.

1. Starting with a confluent layer of MEFs, and working in a tissue culture hood, remove spent medium and rinse the culture several times with sterile Thermo Scientific HyClone ES Qualified DPBS (SH30850.03) to remove traces of serum. Serum inactivates trypsin.
2. Add Thermo Scientific HyClone Trypsin (SH30236.01) or HyQTase (SV30030.01) to cover cells (1-5 mL). If using Trypsin, incubate until the cells detach from the plate (3-5 minutes). If using HyQTase, use at room temperature until the cells detach from the plate (3-5 minutes).
3. Add an equivalent volume of MEF medium (Table 1) and break up cell aggregation by gently pipetting up and down.
4. Plate at  $5.0 \times 10^4$  cells/cm<sup>2</sup> in fresh MEF medium. Passage at a 1:3 split.

### *Related Protocols*

- SC Protocol 00001 - Mouse Embryonic Feeder Cell Protocol: Thawing Cryopreserved MEFs
- SC Protocol 00003 - Mouse Embryonic Feeder Cell Protocol: Mitotic Inactivation of MEFs by Mitomycin C
- SC Protocol 00004 - Mouse Embryonic Feeder Cell Protocol: Cryopreservation of MEFs

### *References:*

Wesselschmidt, R. L. Primogenix, Inc., [robin@primogenix.com](mailto:robin@primogenix.com)

©2009 Thermo Fisher Scientific Inc. All rights reserved.