

# Mouse Embryonic Feeder Cell Protocol: Thawing Cryopreserved MEFs

Protocol  
SC 00001

## 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 200 mM	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 have also 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. MEFs 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

Upon receipt of a frozen vial of MEFs, immediately place vial in liquid nitrogen storage and record the location, source and other relevant information. MEFs should be thawed at least one week before they are needed for co-culture with ESCs (unless thawing previously inactivated MEFs).

### *Thawing Protocol*

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. Recommended seeding density is approximately 40,000-60,000 cells/cm<sup>2</sup>.

1. When MEFs are required for culture, gather culture vessels and prepare media. Warm media to 37°C and place warmed MEF medium in a tissue culture flask.
2. Wearing appropriate protective gear (eye protection and insulated gloves), retrieve a vial of MEFs from cryopreservation and thaw the vial by swirling it in a 37°C water bath—be careful to prevent water from splashing near the cap opening as such splashing may increase the possibility of contamination.
3. Dry the vial with a paper towel and spray it with 70% ethanol.
4. Carefully open the vial and pipette the contents into an empty 15 mL centrifuge tube. Slowly add 5 mL of warmed media (drop wise over about 1 minute) to the tube.
5. Centrifuge at 100 x g for 5 minutes.
6. Decant media from the tube and gently re-suspend the cell pellet in 5 mL of media. Transfer to the tissue culture flask prepared in step 1.
7. Incubate at 37°C and 5% CO<sub>2</sub> overnight.
8. The next day, observe cells under a microscope for attachment and appearance of healthy cells. Remove spent media and replace with fresh MEF medium.
9. Every other day, aseptically remove spent medium from MEF culture and replace with an equivalent volume of fresh MEF medium. Return cells to incubator.
10. Continue until cells become confluent.
11. Passage, inactivate or freeze as required.

### *Related Protocols*

- SC Protocol 00002 - Mouse Embryonic Feeder Cell Protocol: Subculturing 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

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