

# Mouse Embryonic Feeder Cell Protocol: Cryopreservation of MEFs

Protocol  
SC 00004

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

*For research use only*

## *Background*

Mouse embryonic fibroblasts (MEFs) have been used as feeder cell support layers to culture embryonic stem cells (ESCs) since the first mouse ESCs were derived in 1981. They continue to be the most commonly used feeder layer when culturing either mouse or human ESCs. MEFs provide a complex, unknown mixture of nutrients and substrata for long term growth and proliferation of undifferentiated ESCs.

## Required Materials

- Flask of MEFs
- MEF medium (see Table 1)
- Thermo Scientific HyClone MEF cryopreservation medium (SH30894.01; 100 mL)
- Thermo Scientific HyClone HyQTase (SV30030.01) or Thermo Scientific HyClone Trypsin (SH30236.01; 100 mL)
- Thermo Scientific HyClone AdvanceSTEM ES Cell Qualified DPBS (SH30850.03; 1000 mL)
- Thermo Scientific Nalgene “Mr. Frosty” Freezing Container (Fisher catalog no. 15-350-50)
- Cryopreservation vials (Fisher catalog no. 12-565-163N or equivalent)
- General cell culture supplies

## *Media Preparation*

Table 1: MEF Medium

Thermo Scientific HyClone Product	Volume (500 ml final)	Catalog Number
AdvanceSTEM™ DMEM4SC	440 mL	SH30824.01 (500 mL)
ES Screened FBS	50 mL (10%)*	SH30070.03E (500 mL)
AdvanceSTEM ES Qualified L-glutamine 200mM	5.0 mL	SH30852.01 (100 mL)
AdvanceSTEM ES Qualified (NEAA) 100X	5.0 mL	SH30853.01 (100 mL)
Penicillin / Streptomycin Solution (optional)	5.0 mL	SV30010 (100 mL)
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*

When MEFs are properly grown it is easy to expand these cells from passage 2 to 7 with very little loss of cell viability and integrity. Culturing and maintaining MEFs just as carefully as ESCs is important. Unhealthy or overgrown MEF feeders results in unhealthy ESCs. MEFs are primary cells with a limited lifespan in culture. If they begin to elongate and doubling time increases significantly, they are beyond their useful passage number. MEFs should be carefully monitored to avoid overgrowing the culture which results in early senescence.

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

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

*Cryopreservation:*

1. Starting with a confluent flask of MEFs, rinse the cells once with Thermo Scientific HyClone ES Qualified DPBS to remove traces of serum.
2. Add HyQTase to cover cells and allow flask to incubate at ambient conditions (between 15°C and 25°C) for 10 minutes or until the cells are dissociated from the flask and free of clumps. Some agitation may be required to achieve single cell suspension. Alternatively, add Trypsin to cover cells and incubate at 37°C for 5 minutes or until the cells are dissociated from the flask and free of clumps. Some agitation may be required to achieve single cell suspension.
3. If HyQTase was used in step 2, no inactivation at this point is required. If Trypsin was used in step 2, inactivate the Trypsin by adding sufficient volume of MEF medium (Table 1) containing serum to cover the cells and to perform a cell count.
4. Pool cells from multiple flasks to minimize variation.
5. Count viable cells, then centrifuge for 5 minutes at 200xg.
6. Discard supernatant and re-suspend the pellet in AdvanceSTEM Cryopreservation Medium to the desired final freezing concentration. We recommend a final cell concentration of  $1.0 \times 10^7$  cells/mL for dispensing into cryovials. Work quickly to prevent cell damage.
7. Dispense 1.0 mL of cell mixture into pre-labeled cryovials.
8. Place cryovials into a Nalgene Freezing Container which is pre-filled with 250 mL isopropanol and freeze at -80°C overnight (no longer than 18 hours). This ensures that a rate of -1°C/minute cooling occurs to minimize cell damage.
9. After -80°C storage, quickly transfer the cryovials to liquid nitrogen storage (-196°C) for long-term storage. Record the type of cell, location and other relevant information for retrieval.

*Related Protocols:*

- SC Protocol 00001 - Mouse Embryonic Feeder Cell Protocol: Thawing Cryopreserved MEFs
- 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

*References:*

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