

Mouse Embryonic Feeder Cell Protocol: Mitotic Inactivation of MEFs by Mitomycin C

Protocol
SC 00003

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

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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

- Flask of MEFs
- MEF medium (see Table 1)
- MEF inactivation medium (see Table 2)
- 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

Table 2: MEF Inactivation Medium

Product	Volume (200 mL final)	Catalog Number
Thermo Scientific HyClone AdvanceSTEM ES Qualified PBS	5 mL	SH30850.03
Fisher (Arcos) Mitomycin C	2 mg	AC22694-0020
Thermo Scientific HyClone Medium (Table 1)	195.0 mL	See Table 1

Dissolve Mitomycin C in PBS; add to MEF medium and sterile filter using a Thermo Scientific Nalgene FastCap Bottle top filter (Fisher 09-740-68A, or equivalent). Aliquot and freeze into 1 time use volumes. Thaw immediately prior to use. Frozen inactivation medium may be used for up to six months after freezing.

General Considerations

- MEFs require careful culture and maintenance. Keeping MEFs in a healthy proliferating state and producing the entire matrix and growth factor support for ESCs, is an important goal. Since MEFs are primary cells, they have a limited lifespan in culture (1-5 passages). 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
- MEFs must be healthy and actively dividing prior to inactivation and their subsequent use as a feeder layer for ESCs. After the MEFs have been inactivated, replace the MEF media every other day. Inactivated MEFs may be cryopreserved for future use (see SC Protocol Sheet 00004). If inactivated MEFs are fed every other day, they make good feeders for mouse ESCs for 5-7 days. For human ESC culture, it is best to inactivate MEFs the day before passaging the hESCs.
- Do not inactivate MEFs until they are needed. It is not possible to expand mitotically inactivated MEFs.
- Exposure to Mitomycin C at 10 µg/mL for 2-3 hours is sufficient to mitotically inactivate MEFs. Inactivated MEFs are counted and plated on tissue culture vessels to give the desired MEF concentration for the culture of the ESC line.
- Mitomycin C inhibits DNA synthesis and nuclear division. It is toxic and possibly carcinogenic. Therefore handle with caution by covering bare skin and wearing protective gloves and safety glasses. Work in an area with good ventilation. Read MSDS from provider before handling and for proper disposal methods.

Inactivation Protocol

1. Beginning with a confluent layer of MEFs, remove the growth medium from the flask and replace it with 10 mL/75 cm² of recently thawed or freshly prepared inactivation medium (Table 2) to cover the monolayer. Place the flask in incubator for approximately 2-3 hours.
2. Remove inactivation medium from the flask. Properly dispose of the inactivation medium.
3. Wash the monolayer of cells at least three times with a minimum of 10 mL/75 cm² of HyClone ES Qualified DPBS.
4. Add Thermo Scientific HyClone Trypsin (SH30236.01) or HyQTase (SV30030.01) to cover cells (1-5 mLs). 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).
5. Add equal amounts of MEF medium (equal to amounts of Trypsin or HyQTase used) and break up cell aggregation by pipetting up and down.
6. Count cells.
7. Plate 4.0 to 6.0 x 10⁴ cells/cm². For example plate 1 x 10⁶ to 1.5 x 10⁶ cells per T-25 flask.
8. Incubate overnight and use as feeder layers the next day and up to day six. Change medium every other day.

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 00004 - Mouse Embryonic Feeder Cell Protocol: Cryopreservation of MEFs

References:

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