Mouse embryonic feeder cell protocol: mitotic inactivation of MEF cells by mitomycin C

HyClone media and supplements

Overview

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

The procedure is adapted from *Wesselschmidt, R. L. Primogenix, Inc* (1).

Required materials:

- Flask of MEF cells
- MEF medium (See *Table 1, on page 1*)
- MEF inactivation medium (see *Table 2, on page 1*)
- HyClone™ ES Qualified DPBS (SH30850.03)
- HyClone Trypsin (SH30236.01) or HyQTase™ (SV30030.01) cell detachments solutions
- General cell culture supplies

IMPORTANT
FOR RESEARCH USE ONLY

Medium preparation

Prepare MEF cell culture medium according to *Table 1, on page 1*. Aseptically combine medium, fetal bovine serum (FBS), and supplements and mix by gently inverting a closed container. Store medium at 4°C. Unused medium should be discarded after six weeks.

Prepare MEF cell inactivation medium according to *Table 2, on page 1*. Dissolve mitomycin C in phosphate buffered saline (PBS), add to MEF medium, and sterile filter using a bottle top filter. Aliquot and freeze into one-time use volumes. Thaw immediately prior to use. Frozen inactivation medium might be used for up to six months after freezing.

Table 1. Preparation of MEF cell medium

Component	Volume (250 mL final)	Product code
AdvanceSTEM™ DMEM4SC	440 mL	SH30824
HyClone ES Screened FBS	50 mL (10%)[1]	SH30070(E)
HyClone AdvanceSTEM ES Qualified L-glutamine 200 mM	5.0 mL	SH30852
HyClone AdvanceSTEM ES Qualified Non-Essential Amino Acids (NEAA) 100 ×	5.0 mL	SH30853
HyClone Penicillin/Streptomycin Solution (optional)	5.0 mL	SV30010

[1] Good results have also been achieved with 15% FBS.

Table 2. Preparation of MEF inactivation medium

Component	Volume (250 mL final)	Product code
HyClone AdvanceSTEM ES Qualified PBS	5 mL	SH30850.03
Mitomycin C (Sigma M4287 or equivalent	2 mg	AC22694-0020
MEF medium (*Table 1, on page 1*)	195.0 mL	-

General considerations

MEF cells require careful culture and maintenance. Keeping MEF cells in a healthy proliferating state and producing the entire matrix and growth factor support for ESCs, is an important goal. As MEF cells are primary cells, they have a limited lifespan in culture (1 to 5 passages). If the cells begin to elongate and doubling time increases significantly, they are beyond their useful state. The cells need to be carefully monitored to avoid over growing the culture, which can results in early senescence. The recommended criteria when sourcing MEF cells are that the cells should be:

1. Isolated from 12.5 to 13.5 day mouse embryos
2. Mycoplasma free
3. Mouse antibody production (MAP) tested

MEF cells must be healthy and actively dividing prior to inactivation and their subsequent use as a feeder layer for ESCs. After the MEF cells have been inactivated, replace the MEF medium every other day. Inactivated MEF cells may be cryopreserved for future use (see *Procedure Mouse embryonic feeder cell protocol: cryopreservation of MEF cells, 29154591*). If inactivated MEF cells are fed every other day, they make good feeders for mouse ESCs for 5 to 7 days. For human ESC culture, the MEF cells should be inactivated the day before passaging the hESCs.

Do not inactivate MEF cells until they are needed. It is not possible to expand mitotically inactivated MEF cells.

Exposure to mitomycin C at 10 µg/mL for 2 to 3 h is sufficient to mitotically inactivate MEF cells. Inactivated MEF cells 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.

Protocol

Inactivation

Step	Action
1	Beginning with a confluent layer of MEF cells, remove the growth medium from the flask and replace it with 10 mL/75 cm^2 of recently thawed or freshly prepared inactivation medium (*Table 2, on page 1*) to cover the monolayer. Place the flask in incubator for approximately 2 to 3 h.
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 cm^2 of ES Qualified DPBS.
4	Add trypsin or HyQTase to cover cells (1 to 5 mL). If using trypsin, incubate until the cells detach from the plate (3 to 5 min). If using HyQTase, use at room temperature until the cells detach from the plate (3 to 5 min).
5	Add equal amounts of MEF medium to used amount of trypsin or HyQTase and break up cell aggregation by pipetting up and down.
6	Count cells.
7	Plate 4.0 to 6.0 × 104 cells/cm2. For example plate 1 × 106 to 1.5 × 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.

Reference

1. Wesselschmidt, R. L. Primogenix, Inc.

Related procedures

Procedure: Mouse embryonic feeder cell protocol: thawing cryopreserved MEF cells 29154595.

Procedure: Mouse embryonic feeder cell protocol: subculturing MEF cells. 29154593.

Procedure: Mouse embryonic feeder cell protocol: cryopreservation of MEF cells. 29154591.

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