Mouse embryonic feeder cell protocol: subculturing MEF cells

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:

- Vial of cryopreserved MEF cells
- MEF medium (Table 1)
- HyClone™ ES Qualified DPBS (SH30850.03)
- HyClone Trypsin (SH30236.01) or HyQTase™ (SV30030.01) cell detachment solution
- 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

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Medium preparation

Prepare MEF cell culture medium according to Table 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.

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.

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:

- Isolated from 12.5 to 13.5 day mouse embryos
- Mycoplasma free
- Mouse antibody production (MAP) tested

Protocol

Subculturing of MEF cells

Step	Action
1	We recommend following the instructions supplied by the provider of the MEF cells. Alternatively, our standard protocol listed here can be used. In all instances, follow proper aseptic technique and work under appropriate tissue culture hood where applicable.
2	Starting with a confluent layer of MEF cells, and working in a tissue culture hood, remove spent medium and rinse the culture several times with sterile ES Qualified DPBS to remove traces of serum. Serum inactivates trypsin.
3	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).
4	Add equal amounts of MEF medium (Table 1) to used amount of Trypsin or HyQTase and break up cell aggregation by gently pipetting up and down.
5	Plate at 5.0 × 104 cells/cm2 in fresh MEF medium (Table 1). Passage at a 1:3 split ratio.

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: mitotic inactivation of MEF cells by mitomycin C. 29154592

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

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