Applied StemCell, Inc.
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Datasheet

CF1 MEF Cells, P2, untreated
(CF1 Mouse Embryonic Fibroblast Cells)

Product Information

Specifications

Catalog Number	Cells per Vial	Treatment	Number of Vials
ASF-1201	1 x 10^6	Untreated	1
ASF-1202	1 x 10^6	Untreated	3

Description

MEF cells serve as feeder cells that support the growth of undifferentiated mouse or human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs).

MEF cells from Applied StemCell with catalog numbers ASF-1201 and ASF-1202 are isolated from 13.5-day old mouse embryos of a representative cross section of the Carworth CF-1 colony from Charles River. Before use as feeder cells, MEF cells must be mitotically inactivated by γ-irradiation or mitomycin-C treatment.

All MEF Feeder Cells from Applied StemCell are produced with consistency and under rigorous quality control in the United States, in our facilities in Oklahoma and California.

Passage

P2

Treatment

Untreated

Shipping

Dry ice

Storage and Stability

Store in liquid nitrogen freezer immediately upon receipt. This product is stable for at least 6 months from the date of receiving when stored as directed.

Biosafety Level

BSL-1

Safety Precaution

PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. Please wear the appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling the cells. Handle the frozen vials with due caution. Please be aware that the following scenario can occur: Gas or liquid nitrogen can leak into the vials when stored in liquid nitrogen. Upon thawing, the gas phase may expand, resulting in a dangerous build-up of pressure within the vial. This can result in the vial exploding and expelling not only the vial contents but also the vial cap and plastic fragments of the vial.

Warranty

The performance of Applied StemCell's MEF Feeder Cells has been validated with the recommended culture media and reagents. Applied StemCell will not be held responsible if components other than those described in this datasheet are used to culture Applied StemCell MEF Feeder Cells.

Restricted Use

This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

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Media and Material

Table 1. MEF feeder cell culture medium

Component	Concentration
DMEM (High Glucose, contains sodium pyruvate and L-Glutamine)	90%
FBS	10%
Non-essential amino acids	0.1 mM

We recommend supplementing your medium with antibiotics—either penicillin-streptomycin or antibiotic-antimycotic—to prevent bacterial and fungal contamination.

Table 2. Suggested plating density

Dish Size	Surface Area*	Working Volume	MEF per Dish / Well
100 mm	55 cm^2	12 mL	1.38 - 2.75 x 10^6
60 mm	21 cm^2	5 mL	0.53 - 1.05 x 10^6
35 mm	9 cm^2	2 mL	0.23 - 0.45 x 10^6
T25	25 cm^2	5 mL	0.63 - 1.25 x 10^6
T75	75 cm^2	15 mL	1.88 - 3.75 x 10^6
T175	175 cm^2	50 mL	4.38 - 8.75 x 10^6
6-well	9.5 cm^2	2 mL	0.24 – 0.48 x 10^6
12-well	3.8 cm^2	1 mL	0.10 – 0.19 x 10^6
24-well	1.9 cm^2	500 µL	47,500 – 95,000
48-well	0.95 cm^2	250 µL	23,800 – 47,500
96-well	0.32 cm^2	100 µL	8,000 – 16,000

**Approximate growth surface areas. Numbers can vary between plasticware from different suppliers*

Protocol

1. **Apply coating matrix to cell culture vessels**

   1.1 Coat your plates with 0.1% gelatin.

   1.2 Incubate at room temperature for at least 30 minutes before use.

2. **Prepare MEF feeder cell culture medium**

   2.1 Prepare MEF culture medium (Table I), pre-warm the medium at room temperature or in a 37°C water bath before thawing the cryopreserved MEF feeder cells.

   2.2 The culture medium should be aliquoted and stored at -20°C if it will not be used immediately.

   Note: The culture medium can be stored at 4°C for up to 4 weeks or at -20°C for up to 12 months.

3. Thaw and culture cryopreserved MEF feeder cells

3.1 To thaw the cryopreserved MEF feeder cells, remove one vial from the storage unit.

3.2 Immerse the vial in a 37° water bath (up to 2/3rd of the vial) and thaw the cells rapidly until only a small piece of ice is still visible (approximately one minute).

Note: Do not shake the vial during thawing.

3.3 Immediately bring the vial to the biological safety cabinet, spray the outside of the vial thoroughly with sterile 70% ethanol, and wipe it with an sterile wipe.

3.4 Remove the cells from the vial using a p1000 micropipette (or serological pipette) and transfer them slowly, drop-wise while swirling into a 15 mL conical tube containing 5 mL of pre-warmed MEF feeder cell culture medium. Wash the vial with 1 mL medium from the 15 mL conical tube and transfer it back to the tube.

Note: Do not mix cells up and down and avoid generating bubbles.

3.5 Centrifuge cells at 250 x g for 5 minutes at room temperature.

3.6 Very carefully aspirate the medium using a vacuum or pipette if preferred, leaving only a drop of liquid in the tube.

Note: Take extra care not to remove or disturb the cell pellet during aspiration of the medium.

3.7 Using a p1000 micropipette, add 1 mL of the pre-warmed MEF feeder cell culture medium to the tube and gently resuspend cells by pipetting up and down 2-3 times.

3.8 Remove a 10 µL aliquot of the cell suspension and mix it with 10 µL of Trypan blue solution.

3.9 Count the cells.

3.10 Aspirate the coating matrix from the pre-warmed cell culture vessel.

3.11 Seed the MEF feeder cells at a density ranging from 25,000-50,000 live cells/cm^2 (Table II) in MEF feeder cell culture medium. The optimal density is determined by your specific application.

Note: Because we are committed to your success, we are ready to answer any technical questions you may have. Please contact us at info@appliedstemcell.com if you need help determining the optimal density for your application.

3.12 Distribute the cells evenly.

3.13 Place the cell culture vessels into the incubator (37°C/ 5% CO_2/ humidity control) for 2-3 days.

3.14 After 2-3 days (cells should have grown up to 90% confluency), proceed to mitotic inactivation by one of the following methods.

Mitotic inactivation by γ-irradiation

When cells are confluent, trypsinize the cells, spin down, resuspend cells in chilled growth medium, and γ-irradiate the cell suspension at 4000 rad.

Mitotic inactivation by Mitomycin-C treatment

When cells are confluent, treat the cells with 10 µg/ml mitomycin C for 2 hours, then trypsinize the cells, spin down and resuspend in growth medium, and plate for use (cells can also be cryopresereved in freezing medium).

3.15 For use as feeder cells, plate mitotically inactivated cells (see above tables) at an appropriate density in a gelatin-coated tissue-culture dish (generally 25,000-50,000 cells/cm^2, Table 2). Optimal density is to be determined by the user for specific applications.