Applied StemCell, Inc.
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Datasheet

DR4 MEF Cells, P2, Untreated
(4-Drug Resistant Mouse Embryonic Fibroblast Cells)

Product Information

Specifications

Catalog Number	Cells per Vial	Treatment	Number of Vials
ASF-1001	1 x 10^6	Untreated	1
ASF-1002	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 are isolated from 13.5-day old mouse embryos and should be used at early passages. Before use as feeder cells, MEF cells must be mitotically inactivated by γ-irradiation or mitomycin-C treatment.

DR4 MEF cells are derived from mice that are genetically engineered with 4 drug-resistant genes, neomycin, hygromycin, puromycin and 6-thioguanine.

Background: 129P2/OlaHsd, 129S4/SvJae, BALB/c and C57BL/6

Passage

P2

Treatment

Untreated

Shipping

Dry ice

Storage and Stability

Store in vapor phase liquid nitrogen or thaw vial(s) upon arrival to insure the highest viability. Storing at -80°C will result in loss of viability.

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: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, 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.

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

Medium (I)

Component	Concentration	Vendor
ESC-Sure™ DMEM		Applied StemCell, #ASM-5001
ESC-Sure™ FBS	10%	Applied StemCell, #ASM-5007
Nonessential amino acids	0.1 mM	Life Technologies, #11140-050
Sodium Pyruvate	1 mM	Life Technologies, #11360-070
L-Glutamine	2 mM	Life Technologies, #25030-164

Suggested drug concentration for Mouse and Human ES/iPS cell culture (II)

	Mouse ESC/iPSC	Human ESC/iPSC
Neomycin	200 µg/ml	50-150 µg/ml
Puromycin	1 µg/ml	0.5 - 1 µg
Hygromycin	120 µg/ml	10 - 40 µg/ml
6-Thioguanine	1 - 10 µg/ml	1 - 5 µg/ml

Always perform a dose-response curve ("kill-curve") to determine the optimal concentration.

Suggested plating density (III)

Dish Size	Surface Area*	Working volume	MEF per dish / well
100 mm	55 cm²	11 - 16.5 ml	1.7 - 2.8 x 10^6
60 mm	21 cm²	4.2 - 6.3 ml	0.65 – 1.1 x 10^6
35 mm	9 cm²	1.8 - 2.7 ml	0.27 – 0.45 x 10^6
T25	25 cm²	5 – 7.5 ml	0.75 - 1.25 x 10^6
T75	75 cm²	15 – 22.5 ml	2.25 - 3.75 x 10^6
T175	175 cm²	35 – 52 ml	5.25 - 8.75 x 10^6
6-well	9.5 cm²	1.9 - 2.9 ml	0.29 – 0.48 x 10^6
12-well	3.8 cm²	0.8 - 1.2 ml	0.11 – 0.19 x 10^6
24-well	1.9 cm²	0.4 - 0.6 ml	57,000 – 95,000
48-well	0.95 cm²	0.2 - 0.3 ml	22,500 – 47,500
96-well	0.32 cm²	100 - 200 µl	9,600 – 16,000

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

Protocol

1. Remove a vial of frozen cells from liquid nitrogen and place it onto dry ice for 5' before thawing it at 37 ºC water bath. As soon as the majority of the content of the vial thawed, transfer it to a conical tube containing 10x volume of pre-warmed medium.

2. Spin at 1000 rpm for 5 min, discard medium, resuspend the cells in growth medium and plate them at an appropriate density in a gelatin-coated tissue-culture dish (generally 25,000-50,000 cells/cm^2, Media and Material III). Optimal density is to be determined by the user for specific applications.

3. After 2-3 days, trypsinize the cells and subculture at 1:3 ratio.

4. For use as feeder cells, plate mitotically inactivated cells (see below) at an appropriate density in a gelatin-coated tissue-culture dish (generally 30,000-50,000 cells/cm^2, Media and Material III). Optimal density is to be determined by the user for specific applications.

- **Mitotic inactivation by γ-irradiation**
  When cells reach confluency, trypsinize the cells, spin down, resuspend cells in chilled growth medium, and γ-irradatiate the cell suspension at 3000 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 frozen down in freezing medium).