EmbryoMax® PMEF, Strain DR4, Mytomycin C treated, passage 3

Primary Mouse Embryo Fibroblasts

Cat. # PMEF-DR4

Pack size: 1 vial

Store in Liquid Nitrogen

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION

Certificate of Analysis

Background

The EmbryoMax range of PMEF cells provides researchers with a convenient solution for ES cell culture by eliminating the need for time consuming feeder cell isolation and preparation. Many embryonic stem cell culture protocols necessitate the use of primary mouse embryo fibroblast (PMEF) cells. In these protocols, ES cells are typically cultured on a monolayer of PMEF feeder cells. Feeder cells perform two important roles in stem cell culture: they secrete several important growth factors into the medium, which help maintain pluripotency, and they provide a cellular matrix for ES cells to grow.

The DR4 strain of MEF feeder cells are resistant to neomycin, hygromycin, puromycin, and 6-thioguanine. They are mitotically arrested by mytomycin-C treatment and will not proliferate.

Jaenisch et. al, *A transgenic mouse strain expressing four drug-selectable marker genes.* Nucleic Acids Res. 1997 Sep 15; 25(18): 3745–3746.

Cells originally manufactured by Applied Stem Cell, Inc.

Components

EmbryoMax PMEF, P3, Strain DR4, Mito-C Treated: (Part number PMEF-DR4) One (1) vial containing ≥4 x10^6 cells per vial.

Storage Conditions

Vials should be stored in the vapor phase of liquid nitrogen.

Protocol

Count cells prior to plating. Plate the PMEF feeder cells one day prior to plating ES cells, targeting approximately 95% confluence. If ES cells are plated earlier than one day after PMEF plating, there may be some small gaps in the feeder layer. Although plating ES cells when gaps are present may not have any detrimental effect on the ES cells, it is not recommended.

1. Prior to thawing PMEF feeder cells, coat plates / flasks with Gelatin solution (Cat. No. ES-006-B):
   a. Warm 0.1% Gelatin Solution to room temperature prior to use.
   b. In a culture hood add enough Gelatin Solution to adequately cover the plasticware surface, approximately 3 mL per 25 cm^2.
   c. Leave the Gelatin Solution in the wells for a minimum of 30 minutes at room temperature, with dish lids on, in the laminar flow hood.

Protocol (continued)

2. Thaw PMEF vial(s) quickly in a 37°C water bath and transfer to a 15 mL tube containing 10 mL of warm PMEF Feeder Cell medium (Table 1). Gently invert the tube, and centrifuge at 300 xg for 4-5 minutes.
3. Remove supernatant and resuspend the cell pellet in PMEF Feeder Cell Medium.
4. Remove the Gelatin solution from plates / flasks, and aliquot the PMEF feeder cell suspension at the densities recommended below (Table 2). For human ES cell culture, refer to Table 3 for recommended plating densities.
5. Incubate the PMEF Feeder cells at 37°C with 5% CO_2. Inactivated feeder cells plated on gelatinized plates may be used for 12-14 days.

Table 1. PMEF Culture Medium

Component	Millipore Cat. No.	% (v/v)
DMEM, low bicarbonate	SLM-220-B	N/a
ES Cell Qualified FBS	ES-009-B	10%
Penicillin-Streptomycin	TMS-AB2-C	1%
L-Glutamine Solution (100x)	TMS-002-C	1%

Table 2. Plating densities for mouse ES cell culture

Dish size	Volume	Growth Area	Cell Density
75 cm^2 flask	12 mL	75 cm^2	3.75 x 10^6
25 cm^2 flask	6 mL	25 cm^2	1.25 x 10^6
100 mm plate	10 mL	56 cm^2	2.8 x 10^6
60 mm plate	5 mL	21 cm^2	1.0 x 10^6
6-well plate	4 mL	9.5 cm^2	4.75 x 10^5
12-well plate	2 mL	4 cm^2	2.0 x 10^5
24-well plate	1 mL	2 cm^2	1.0 x 10^5
96-well plate	0.1 mL	0.32 cm^2	1.5 x 10^4

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

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Table 3. PMEF-CFX plating densities for human ES cell culture

Dish size	Volume	Growth Area	Cell Density
75 cm^2 flask	12 - 15 mL	75 cm^2	$9.3 \times 10^5 – 1.3 \times 10^6$
25 cm^2 flask	5 - 7 mL	25 cm^2	$3.1 \times 10^5 – 4.7 \times 10^5$
100 mm plate	6 - 8 mL	56 cm^2	$7.0 \times 10^5 – 1.0 \times 10^6$
60 mm plate	4 - 7 mL	21 cm^2	$2.6 \times 10^5 – 3.9 \times 10^5$
6-well plate	2.5 - 4 mL	9.5 cm^2	$1.2 \times 10^5 – 1.8 \times 10^5$
12-well plate	2 mL	4 cm^2	$5.3 \times 10^4 – 8.0 \times 10^4$
24-well plate	1 mL	2 cm^2	$2.6 \times 10^4 – 4.0 \times 10^4$

Note: The lower density is recommended for Mitomycin-C treated feeder cells and the higher value is recommended for irradiated feeder cells.

Quality Control Testing

Cell Viability and Morphology: PASSED

Mycoplasma Testing: PASSED

Bacterial Testing: PASSED

Fungi Testing: PASSED

RELATED PRODUCTS

cat #	description
PMEF-CFL	EmbryoMax PMEF, Strain CF1, Untreated
PMEF-CF	EmbryoMax PMEF, Strain CF-1, Mitomycin C Treated
PMEF-CF-P1	EmbryoMax PMEF, Strain CF1, Untreated, Passage 1
PMEF-CFX	EmbryoMax PMEF, Strain CF-1, Irradiated
PMEF-H	EmbryoMax PMEF, Hygromycin Resistant, Mitomycin C Treated
PMEF-HL	EmbryoMax PMEF, Hygromycin Resistant, Untreated
PMEF-N	EmbryoMax PMEF, Neomycin Resistant, Mitomycin C Treated
PMEF-NL	EmbryoMax PMEF, Neomycin Resistant, Untreated
PMEF-NL-P1	EmbryoMax PMEF, Neomycin Resistant, Untreated, Passage 1
ESG1107	ESGRO® mLIF Medium Supplement, 10^7 units
SLM-220-B	EmbryoMax DMEM (1X), with 4,500mg/L Glucose, 2.25g/L Sodium Bicarb, without L-Glut and Sodium Pyruvate
ES-009-B	EmbryoMax Fetal Bovine Serum
TMS-AB2-C	EmbryoMax Penicillin-Streptomycin Solution, 100X
TMS-002-C	EmbryoMax L-Glutamine Solution (100X), 200mM
TMS-001-C	EmbryoMax MEM, Non Essential Amino Acids (100X)
ES-008-D	EmbryoMax Nucleosides (100X)
ES-007-E	EmbryoMax 2-Mercaptoethanol (100X)
BSS-1005-A	Dulbecco's Phosphate Buffered Saline (1X)

GMO
This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement des modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.