

Product Specification

Item Name:

Irradiated C57BL/6J-Tg(pPGKneobpA)3Ems/J (B6-Neo^R) JAX® MEFs

Organism: *Mus musculus* (mouse)

Strain Name: C57BL/6J-Tg(pPGKneobpA)3Ems/J

Stock Number: 002356

Number of cells: 5 million per vial

Item Number: 002356M01

Origin: The Jackson Laboratory

Cell type: Fibroblast/MEF

Source: Embryo (12.5 – 13.5 dpc)

Gender: Mixture of males and females

Passage Number: 3

Storage: liquid nitrogen

Quality Assurance: JAX® MEFs are tested for bacterial and fungal growth and for the presence of mycoplasma using a PCR detection system. Cell culture contaminants were not detected. The genotype of the cells was confirmed by SNP analysis.

Comments: The cell culture was established by dissociating embryos (after removal of the head and viscera) from strain C57BL/6J-Tg(pPGKneobpA)3Ems/J (Stock Number 002356) using 0.05% Trypsin/0.02% EDTA in HBSS. JAX® MEFs were passaged twice before freezing. Irradiated JAX® MEFs can be used as a feeder layer to support the growth and maintenance of undifferentiated ES cells.

MEF culture medium

DMEM	500 ml	Sigma #5671
Fetal Bovine Serum (FBS) 10%	57 ml	Biowhittaker #14-501F
Glutamax 2 mM	5.7 ml	Invitrogen #35050-061 (100x)
Sodium pyruvate (1 mM)	5.7 ml	Invitrogen #11360-070 (100x)
Pen-Strep (100 units-100 µg/ml)	5.7 ml	Invitrogen #15140-122 (100x)
Non essential aa 0.10 mM	5.7 ml	Invitrogen #11140-050 (100x)
Monothioglycerol (MTG) 150 µM	0.5 ml working dilution*	Sigma #M6145

*Prepare working daily dilution by adding 27 µl stock MTG into 2 ml DMEM, mix.

Thaw and culture protocol

1. One vial of cells (5×10^6 cells/vial) will be a quantity sufficient to cover 1 x T₇₅ tissue culture flask, 3 x T₂₅ tissue culture flasks or 2 x 6-well plates.
2. Thaw the vial rapidly in 37° C water bath.
3. Transfer contents to 15 ml sterile conical tube.
4. Slowly add 10 ml MEF medium with mixing.
5. Centrifuge tube at 1200 rpm for 4 minutes in a table top centrifuge.
6. Aspirate and discard supernatant. Vortex pellet and suspend in 15 ml MEF medium, transfer to tissue culture flask(s).
7. Incubate in 95% air; 5% CO₂ at 37° C and allow overnight attachment.
8. Next morning, remove supernatant and replace with fresh culture medium.