

Product Specification

Item Name: 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: 002356M00

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. It is recommended that JAX[®] MEFs not be used beyond passage 6-8. The recommended split ratio for passaging is approximately 1:3.

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.

Cryopreservation Medium

90% FBS and 10% DMSO, prepare in quantities that can be used in a 2-week period.

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.

Passaging non-irradiated JAX[®] MEFs

1. Remove and discard the medium from the tissue culture flask.
2. Rinse the adherent cells with small amount of 0.05% Trypsin/0.02% EDTA to remove traces of serum.
3. Add a quantity of 0.05% Trypsin/0.02% EDTA to cover the cell layer, usually 1/3 the amount used to culture the cells.
4. Incubate cells with 0.05% Trypsin/0.02% EDTA for 5-7 minutes, watching for cell lift using the inverted microscope.
5. Collect the cells in 0.05% Trypsin/0.02% EDTA and then rinse the culture dish with an equivalent amount of MEF medium and collect both aliquots in one tube.
6. Centrifuge tube contents, count and suspend cells in freezing medium, if freezing cells, or in culture medium at a ratio of 1:3 if expanding cells in culture.
7. Incubate in 95% air; 5% CO₂ at 37° C until 90-95% confluency.