JAX® Mouse Embryonic Stem Cells
C57BL/6J-693 mES cells

Product Specification

Item Name:	C57BL/6J-693 mES cells	**Donating Investigator**:	K. Schuster-Gossler
Item Number:	000664C01	**Cell Type**:	Undifferentiated mES Cells
Stock Number:	000664	**Source**:	Day 3.5 blastocyst
Organism:	*Mus musculus* (mouse)	**Passage Number:**	11
Strain of Origin:	C57BL/6J (000664)	**Gender**:	Male
Number of Cells:	3 million cell per vial	**Storage**:	Liquid nitrogen

Description

The C57BL/6J-693 mES cell line was established using day 3.5 blastocysts from C57BL/6J (Stock Number 000664) blastocysts†. When "black" C57BL/6J-693 mES cells are injected into "white" B6(Cg)-*Tyr*$^{c-2J}$/J (000058) blastocysts and implanted in pseudopregnant females, chimeric offspring are easily detected by coat color. The B6(Cg)-*Tyr*$^{c-2J}$/J (B6 albino) strain carries a mutation in the tyrosinase gene (*Tyr*$^{c-2J}$) causing complete absence of pigment in homozygous mice.

mES cells are cultured in mES cell medium as described below and replenished daily. mES cells are maintained on irradiated JAX® MEFs from C57BL/6J or B6D2F1/J mice. Therefore, each vial of mES cells contain a small number of irradiated fibroblast feeder cells.

† Schuster-Gossler K, Lee AW, Lerner CP, *et al.* 2001. Use of coisogenic host blastocysts for efficient establishment of germline chimeras with C57BL/6J ES cell lines. *Biotechniques* 31:1022-1024, 1026.

Quality Assurance

JAX® mES cells 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 cell line was confirmed by SNP analysis. mES cells will spontaneously differentiate in the absence of mouse embryonic fibroblasts (MEFs) and supplemental growth factors, which together act as inhibitors of differentiation. C57BL/6J-693 mES cells were proven germline competent at various passages (P13, 15, 19, 31 and 32).

mES Cell culture medium

Prepare in small batches, e.g. 100 ml

Reagent	Volume	Supplier
DMEM high glucose, 4500 mg/L	81 ml	Sigma #5671
Fetal Bovine Serum (FBS) 15%	15 ml	Biowhittaker #14501F
Glutamax, 2 mM	1.0 ml	Invitrogen #35050-061 (100x)
Sodium pyruvate, 1 mM	1.0 ml	Invitrogen #11360-070 (100x)
Pen-strep, 100 units-100 µg/ml	1.0 ml	Invitrogen #15140-122 (100x)
Non essential aa, 0.10 mM	1.0 ml	Invitrogen #11140-050 (10 mM 100x)
Leukemia Inhibitory Factor (LIF) 10^3 units/ml	10 µl	Chemicon ESGRO #ESG-1107
PD 98059, 25 µM	50 µl (stock solution)*	Calbiochem #513000
Monothioglycerol (MTG) 150 µM	100 µl (working dilution)**	Sigma #M6145

* Add 370 µl DMSO to 5mg of PD 98059 to make a 50 mM stock solution. Use of inhibitor is optional.
** Prepare working 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 of JAX® mES cells

1. Plate JAX® MEF feeder cells one day in advance of thawing and plating the mES cells. When plating JAX® MEFs at a concentration of 5 x 10^6 cells/vial, they will cover 1 x T_{75} tissue culture flask, 3 x T_{25} tissue culture flasks or 2 x 6-well plates.
2. Thaw the vial of JAX® mES cells rapidly in a 37° C water bath.
3. Transfer contents to a 15 ml sterile conical tube.
4. Slowly add 5 ml mES cell medium with mixing.
5. Centrifuge tube at 1200 rpm for 5 minutes in a table top centrifuge.
6. Aspirate and discard supernatant. Vortex pellet and suspend in 2 ml mES cell medium, count mES cells and add to a MEF monolayer
   (0.5-1 x 10^6 mES cells per 1 x 10^6 MEFs). Allow mES cells to attach overnight in 95% air; 5% CO_2 at 37° C.
7. Next morning, remove medium and replace with fresh mES culture medium.

Subculturing JAX® mES cells at a ratio of 1:3 to 1:5

1. Remove and discard the mES cell culture medium.
2. Rinse the adherent mES and MEF cells with small amount of 0.05% Trypsin/0.02% EDTA to remove traces of medium and 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 identical amount of mES cell medium. Collect both aliquots (trypsinized cells and rinse) in one tube.
6. Centrifuge tube contents, suspend in either freezing medium for cryopreservation or in culture medium and transfer to a new culture vessel that contains a monolayer of feeder cells. Incubate in 95% air; 5% CO_2 at 37° C, change medium daily.