JAX® Mouse Embryonic Stem Cells
WSB/EiJ-AC635/GrsrJ mES cells

Item Name:	WSB/EiJ-AC635/GrsrJ mES cells
Item Number:	064079
Stock Number:	001145
Organism:	Mus musculus (mouse)
Strain of Origin:	WSB/EiJ
Number of Cells:	3-5 million cells per vial
Donating Investigator:	Laura Reinholdt
Cell Type:	Undifferentiated mES cells
Source:	Day 3.5 blastocyst
Passage Number:	9
Gender:	M
Storage:	Liquid nitrogen

Description

The AC635/GrsrJ embryonic stem cell line was established using day 3.5 blastocysts from WSB/EiJ (Stock Number 001145). The cells were cultured in medium as described below and replenished daily. The cells were maintained on mitomycin-C treated (or otherwise mitotically inactivated) mouse embryonic fibroblast feeder cells (MEFs) derived from C57BL/6J embryos.

Quality Assurance

AC635/GrsrJ was tested for bacterial and fungal growth and for the presence of mycoplasma using a PCR detection system. Cell culture contaminants were not detected. The genetic background of this cell line was confirmed by SNP panel analysis. AC635/GrsrJ was proven germline competent at passage 4. JAX makes no guarantee that this cell line can be used for gene targeting or that if targeted clones are created, they will be germ line competent. mES cells will spontaneously differentiate in the absence of mouse embryonic fibroblasts (MEF) and supplemental growth factors, which together act as inhibitors of differentiation.

Component	Volume	Vendor	Catalog number
DMEM high glucose	81 mls	Invitrogen	11960069
Fetal Bovine Serum (FBS)	15 mls	Lonza	14-501F
GlutaMAX-1	1ml	Invitrogen	35050061
PenStrep	1ml	Invitrogen	15140122
MEM NEAA	1ml	Invitrogen	11140050
Sodium Pyruvate 100mM	1ml	Invitrogen	11360070
2-Mercaptoethanol, 55mM	180 ul	Invitrogen	21985-023
Leukemia inhibitory factor (LIF) 10^7 units	10 ul	Millipore	ESG1107
PD0325901	10 ul (stock solution)*	Stemgent	04-0006
CHIR99021	10 ul (stock solution)**	Stemgent	04-0004

*Add 414 ul DMSO to 2mg of PD0325901 to make a 10mM stock solution. Avoid repeated freeze-thaws.
**Add 140ul DMSO to 2mg of CHIR99021 to make a 30mM stock solution. Avoid repeated freeze-thaws.

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Technical Information Services

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Additional Culture Components

Component	Vendor	Catalog number
PBS pH 7.2, no Ca or Mg	Invitrogen	20012027
0.05% Trypsin-EDTA	Invitrogen	25300054
DMSO	Sigma	D2650

Cryopreservation Medium

80% culture medium, 10% FBS and 10% DMSO, prepare in quantities that can be used within a 2-week period

Thaw and Culture of mES cells

1. Day 0. Plate mitomycin-C MEF (MEFs that are mitotically inactivated by irradiation may also be used) feeder cells on one 60 mm plate (per vial of ES cells) one day in advance of thawing and plating mES cells.

2. Day 1. Thaw one vial of mES cells rapidly in a 37°C water bath. Sterilize vial with 70% ethanol and then transfer cells to 10 mLs of pre-warmed (37°C) media. Centrifuge the cells for 5 minutes at 1000 rpm. Resuspend the pellet in 5 mLs of media and create a single cell suspension by gentle trituration with a serological pipet. Remove MEF media from the 60mm MEF-seeded dish and replace with the ES cell suspension.

3. Day 2. Examine the cells under phase-contrast microscopy. Colonies should be visible and nicely distributed on the MEF feeder layer. Depending on the size and density of the colonies, either replace the culture medium to feed the growing cells or passage the cells 1:3 to 1:4.

4. Passaging mES cells at a ratio of 1:3 to 1:4: Remove and discard the ES cell culture medium. Rinse the cells with room temperature PBS. Add a quantity of .05% Trypsin-EDTA to cover the cell layer, usually 1/3 the amount used to culture the cells. Return the cells to the incubator for 4-6 minutes. Observe cell dissociation using the microscope, with occasional swirling. As soon as colonies begin to dissociate, inactivate the trypsin by adding an equal amount of ES cell medium. Collect the cells and then remove any remaining cells by rinsing the culture dish with additional ES cell medium. Collect both aliquots (trypsinized cells and the rinse) in one tube and create a single cell suspension by gentle trituration with a serological pipet. Centrifuge for 5 minutes at 1000 rpm and resuspend the pellet, creating a single cell suspension by gentle trituration with a serological pipet in either cryopreservation medium or in culture medium and transfer to a new culture plate that contains a feeder layer of mitomycin-C treated MEFs.

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