Mouse Embryonic Stem Cells

The Jackson Laboratory

NOD/ShiLtJ AC576/GrsJ mES cells

Item Name	NOD/ShiLtJ AC576/GrsJ mES cells
Stock Number	026874
Organism	*Mus musculus* (mouse)
Strain of Origin	NOD/ShiLtJ (001976)
Number of Cells	2-4 x 10^6 cells/vial
Donating Investigator	Genetic Resource Science, The Jackson Laboratory
Cell Type	Undifferentiated mES Cells
Source	Day 3.5 blastocyst
Passage Number	5
Gender	Male
Storage	Liquid nitrogen

Description

The AC576/GrsJ NOD cell line was established using day 3.5 blastocysts from pooled JAX GSP stock of NOD/ShiLtJ (Stock No. 001976) when the mice were at generation F121-F126. The cells are cultured in medium as described below and replenished daily. The cells are maintained on mitomycin-C treated fibroblast feeder cells derived from C57BL/6J mice.

Quality Assurance

AC576/GrsJ 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 genotype of the cell line was confirmed by SNP analysis. AC576/GrsJ mES cells will spontaneously differentiate in the absence of mouse embryonic fibroblasts (MEF) and supplemental growth factors, which together act as inhibitors of differentiation. Moreover, these ES cells were created and are typically cultured in the absence of serum. The culture conditions provided below are the standard serum free conditions used to maintain these cells. AC576/GrsJ was proven germline competent at passage 4. **JAX makes no guarantee that this cell line can be used to generate targeted clones or, if targeted clones are created, that they will be germ line competent.**

mES Defined Cell Culture Medium for AC576/GrsJ

See Ying and Smith 2003 [PMID: 14696356]; Ying et al. 2003 [PMID: 12524553]; Ying et al. 2008 [PMID: 18497825]

Stock media

Component	Volume	Vendor	Catalog number
DMEM/F12	495ml	Invitrogen	11320-033
N-2 supplement	5ml	Invitrogen	17502-048
Neurobasal Medium	490ml	Invitrogen	21103-049
B-27 supplement	10ml	Invitrogen	17504-044

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Culture medium (100mls)

Component	Volume	Vendor	Catalog number
DMEM/F12 with N-2 supplement	49 ml	See above	See above
Neurobasal medium with B-27 supplement	49 ml	See above	See above
GlutaMAX-I (100x)	500 ul	Invitrogen	35050061
PenStrep (100x)	1 ml	Invitrogen	15140122
MEM NEAA (100x)	1 ml	Invitrogen	11140050
Sodium Pyruvate 100mM	500 ul	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

Upon preparation, media is filtered through a .22um polyethersulfone filter.
*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.

Additional Culture Components

Component	Vendor	Catalog number
PBS pH 7.2, no Ca or Mg	Invitrogen	20012027
0.05% Trypsin-EDTA	Invitrogen	25300054
Defined Trypsin Inhibitor	Invitrogen	R-007-100
DMSO	Sigma	D2650

Cryopreservation Medium

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

Thaw and culture of mES cells

1. Day 0. Plate low passage* 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 37C water bath. Sterilize vial with 70% ethanol and then transfer cells to 10 mLs of pre-warmed (37C) 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 2-5 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 defined trypsin inhibitor. 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 or to a cryovial.
*MEF viability is highly variable in the defined culture conditions used for this ES cell line. We recommend that MEF viability in these culture conditions be empirically tested prior to culture. Generally, we have found that low passage MEFs are more tolerant to the serum free conditions that we recommend for this ES cell line.

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