PRODUCT DESCRIPTION

CD-1 Mouse Embryonic Fibroblasts (MEFs) can be used as feeder cells for the maintenance of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) in the undifferentiated state. The cells must be mitotically inactivated by irradiation or mitomycin C treatment prior to forming feeder layers.

RECOMMENDED FOR

For the generation of feeder layers for the maintenance of undifferentiated ESCs and iPSCs.

COMPONENTS

Each vial contains 1 x 10⁶ cells in 1 mL in 50% fetal bovine serum and 10% dimethyl sulfoxide.

MEF Catalog # 00321 were prepared from day 12.5 post coitus CD-1 embryos.

MEF Catalog # 00322 were prepared from day 14.5 post coitus CD-1 embryos.

STABILITY AND STORAGE

Store at -135°C or colder, or in liquid nitrogen. Product is stable for a minimum of 6 months from date of receipt when stored correctly.

HANDLING AND DIRECTIONS FOR USE

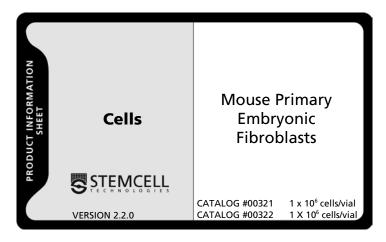
For complete directions for use, please refer to the Technical Manual entitled "Maintenance of mESCs & miPSCs Using ES Cult®", Catalog #29141.

It is advisable to thaw and use cells immediately upon receipt for optimal viability.

All procedures should be carried out using sterile technique in a certified biological safety cabinet.

MEF can be passaged a limited number of times. Slow growth and a 'stringy' appearance are signs of senescence. Therefore, newly acquired MEF should be expanded and multiple vials frozen. Alternatively, MEF can be expanded, irradiated and then frozen. Irradiated MEF can be used as feeder cells one day after thawing and plating.

With the exception of monothioglycerol (MTG), all components essential for the maintenance of mESCs and miPSCs are available as pre-screened ES-Cult® products from STEMCELL Technologies (ES-Cult Complete Maintenance kit, Catalog #03160). If non-ES-Cult® products are substituted, it is essential that they are pre-tested to ensure their ability to maintain mESCs or miPSCs in the undifferentiated state.



REQUIRED MATERIALS

PRODUCT	UNIT SIZE	CATALOG #
ES-Cult® FBS (for mESC/miPSC maintenance)	100 mL 500 mL	06902 06952
Gelatin	500 mL	07903
DMEM high glucose	500 mL	36250
DMEM/F-12	500 mL	36254
Dulbecco's Phosphate Buffered Saline (D-PBS), Mg ⁺⁺ and Ca ⁺⁺ free	500 mL	37350
Trypsin-EDTA	500 mL	07901
L-glutamine	100 mL	07100
Penicillin-Streptomycin (100X)	100 mL	07500
ES-Cult® Complete Maintenance Kit		03150
"Maintenance of mESCs & miPSCs using ES-Cult [®] " Technical Manual		29141

FOR IN VITRO RESEARCH USE ONLY. NOT FOR DIAGNOSTIC, THERAPEUTIC ORCLINICAL APPLICATIONS. NOT APPROVED FOR HUMAN OR VETERINARY USE IN VIVO.

DECEMBER 2010



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WARNING

We do not recommend the storage of frozen cell products in the liquid phase of a liquid nitrogen storage tank. Liquid can enter closed screw top cryovials, which then have the potential to explode due to internal pressure when removed from storage.

Universal handling precautions for biological samples should be used. For more information, please see your site Safety Officer or contact us at techsupport@stemcell.com.

RECOMMENDED FROZEN CELL STORAGE CONDITIONS

For short-term storage (<1 month), store cells in -80°C freezer.

For long-term storage (>1 month), store in the vapor phase of a liquid nitrogen storage tank.

STORAGE PRECAUTIONS

WARNING: We do not recommend the storage of frozen cell products in the liquid phase of liquid nitrogen (LN2). Liquid can enter closed screw top cryovials, which then have the potential to explode when removed from storage.

Our warranty does not cover any losses or damages of any kind due to storage of products in the liquid phase of LN₂.

Laboratory personnel should use extreme caution when storing samples in LN₂. LN₂ storage consists of a liquid phase and a gaseous phase. If cryovials are immersed in the liquid phase, LN₂ can enter the closed screw-top cryovials during storage. The cryovial may then explode when it is removed from storage due to the vaporization and expansion (700x expansion ratio) of the liquid nitrogen inside the cryovial.

HEALTH HAZARDS OF LIQUID NITROGEN

Liquid nitrogen has a 700x expansion ratio, which may cause physical hazards and injuries due to the explosion of cryovials, containers, equipment, or other devices. Extensive tissue damage or burns can result from exposure to LN₂ or cold nitrogen vapors. Asphyxiation may result from the displacement of oxygen in the air with nitrogen to levels where there is insufficient oxygen. Inhalation of oxygen deficient air can cause dizziness, nausea, vomiting, loss of consciousness, and death.

PERSONAL PROTECTIVE EQUIPMENT

The following personal protective equipment is recommended when handling or using LN₂:

Cryo gloves/Waterproof thermal insulated gloves

· Hands should be protected with waterproof thermal insulated gloves that can be quickly removed if LN₂ is spilled on them. These gloves are not intended for submersing hands into LN₂.

Body must be protected with pants, lab coats, and closed-toe shoes.

Face Shield

Eyes are sensitive to the extreme cold of LN₂ and its vapors. Over-pressurization may result in the explosion of improperly stored cryovials. Chemical splash face shields should be used when handling LN₂ and when handling cryovials and other sealed containers that have been stored in LN₂.

The handling of cryovials inside of Biological Safety Cabinets (with the sash lowered) will further reduce the risk of injury from explosions caused by excess pressure within the vial. We recommend that excess pressure be relieved by briefly opening the cap of the cryovial a quarter turn before resealing. This should be done inside a Biological Safety Cabinet.

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