

## RECOMMENDED FOR

The generation of hygromycin-resistant feeder layers for the culture and selection of transfected undifferentiated embryonic stem cells (ESC), where primary mouse embryonic fibroblasts (MEF) and targeted ESC can be co-cultured using hygromycin as a selective agent.

## PRODUCT DESCRIPTION

Hygromycin-resistant MEF are prepared from day E13.5 post-coitus mouse embryos, obtained from female mice (B6D2F1, B6SJLF1 or C3FeB6F1) crossed with transgenic C57BL/6-TgN (pPWL512hyg) mice containing the hygromycin-resistance gene.

MEF are used as feeder cells for the maintenance of mouse ESC in the undifferentiated state. Cells must be mitotically inactivated by irradiation or mitomycin C treatment prior to forming feeder layer for ESC. These MEF are able to maintain undifferentiated ESC while under selection for a hygromycin-resistance marker that has been successfully incorporated into the ESC. MEF are resistant to at least 140 µg/mL hygromycin.

MEF are supplied at passage 1 and can be passaged only a limited number of times (6 – 8 further passages). Slow growth and 'stringy' appearance are signs of senescence. It is recommended that newly acquired MEF be expanded and frozen in multiple vials for future use. Alternatively, MEF can be expanded, mitotically inactivated and frozen. Mitotically inactivated MEF can be used as feeder cells 1 day after thawing and plating.

## COMPONENTS

Each vial contains  $3 \times 10^6$  cells in 1 mL of 95% fetal bovine serum (FBS) and 5% dimethyl sulfoxide.

## 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.

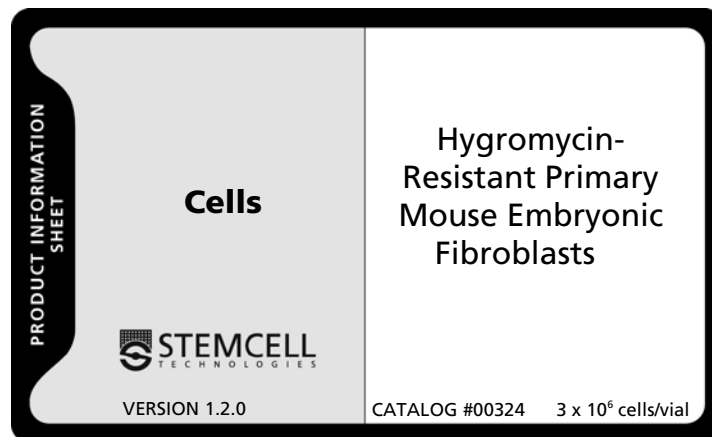
## DIRECTIONS FOR USE

The instructions contained in this protocol assume ESC & iPSC-screened ES-Cult® products from STEMCELL Technologies are used. Other reagents may be substituted, but non-ES-Cult® reagents should be tested to ensure they support ESC & iPSC maintenance and/or in vitro differentiation, as appropriate.

For additional protocols see the Technical Manual for "Maintenance of mESCs & miPSCs using ES-Cult®" (Catalog #29141), available at [www.stemcell.com](http://www.stemcell.com)

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.*



## 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 <sup>++</sup> and Ca <sup>++</sup> 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 OR CLINICAL APPLICATIONS. NOT APPROVED FOR HUMAN OR VETERINARY USE IN VIVO.

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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 (LN<sub>2</sub>). 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 LN<sub>2</sub>.

Laboratory personnel should use extreme caution when storing samples in LN<sub>2</sub>. LN<sub>2</sub> storage consists of a liquid phase and a gaseous phase. If cryovials are immersed in the liquid phase, LN<sub>2</sub> 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 LN<sub>2</sub> 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 LN<sub>2</sub>:

Cryo gloves/Waterproof thermal insulated gloves

- Hands should be protected with waterproof thermal insulated gloves that can be quickly removed if LN<sub>2</sub> is spilled on them. These gloves are not intended for submersing hands into LN<sub>2</sub>.

Clothing

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

Face Shield

- Eyes are sensitive to the extreme cold of LN<sub>2</sub> and its vapors. Over-pressurization may result in the explosion of improperly stored cryovials. Chemical splash face shields should be used when handling LN<sub>2</sub> and when handling cryovials and other sealed containers that have been stored in LN<sub>2</sub>.

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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