OriCell™ KO-Certified 3 Drug-Resistant Mouse Embryonic Fibroblasts, Irradiated

Product Name	OriCell™ KO-Certified 3R MEF
Catalog No.	MUDEF-01002
Product Size	1×10⁶ Cells/ Vial
Passage Number	1
Storage	Liquid Nitrogen

PRODUCT OVERVIEW

Cyagen OriCell™ KO-certified 3R MEF are derived from 13.5-day old mouse embryos and have been genetically engineered to carry 3 genes that confer neomycin, hygromycin, and puromycin resistance. The cells are grown as a monolayer and cryopreserved following mitotic-arrest with γ-irradiation at first passage (P1).

MEF cells are widely used as a feeder substrate for supporting the growth and maintenance of both mouse and human Embryonic Stem Cells (ESCs) in cell culture. Each lot of OriCell™ KO-certified 3R MEF has been tested to effectively maintain pluripotency and the undifferentiated state of human and mouse ESCs. In addition, OriCell™ KO-certified 3R MEF are used as a feeder substrate for ESCs used in Cyagen gene targeting projects, leading to successful generation of knockout and knockin animals.

Additionally, the cells have greater than 80% post-cryopreservation viability and are free of bacteria, fungi, mycoplasma and endotoxin contamination.

HIGHLIGHTED PRODUCT FEATURES

- Mitotically inactivated via γ-irradiation
- Certified to support knockout ES cell or animal generation
- Effectively maintain cellular morphology and attributes of human and mouse ESCs
- Neomycin (≥200μg/ml), Hygromycin (≥60μg/ml), and Puromycin (≥0.4μg/ml) resistant

GENERAL HANDLING PRINCIPLES

- Use aseptic technique when handling this product to prevent microbial contamination
- Cells should be stored at -80°C until use and plated immediately prior to ESC culture
- Cells remain viable for 2 weeks after plating and should be used as soon as possible once thawed

CAUTION: **The freezing medium for this product contains Dimethyl Sulfoxide (DMSO), which may potentially pose hazardous health effects. Please observe your institutional Environmental Health and Safety Protocols when handling the product and follow all published U.S EPA guidelines for proper waste disposal.**

Disclaimer: **This product is intended for laboratory use only.**

Protocol

I. Gelatin Coating of Tissue Culture Vessels

Materials Needed

- 0.1% gelatin solution (Cat. No. GLT-11301)
- 6-well tissue culture plates or vessel of other appropriate sizes

Procedure

1. Add sufficient 0.1% Gelatin Solution into the culture vessel and swirl the plate gently until the solution covers its entire base. Let sit for 30 minutes at room temperature.
2. Aspirate off all the gelatin solution and allow the residual to evaporate by leaving the vessel sitting open in a biological safety cabinet. Limit the evaporation process to no more than 30 minutes.
3. Enclose the culture vessel once it has dried and store at 4°C for no more than 2 weeks to keep sterile.

II. Thawing and Plating OriCell™ KO-Certified 3R MEFs, Irradiated

Materials Needed

- Gelatin-coated 6-well tissue culture plates (or other appropriate sizes)
- OriCell™3 drug-Resistant MEFs (Irradiated) (Cat. No. MUDEF-01002)
- OriCell™ Mouse Embryonic Fibroblast Growth Medium (Cat. No. MUXEF-90011)
- 15 mL aseptic centrifuge tubes
- 37°C water bath
- Sterile pipettors and tips

Procedure

1. Prior to plating, warm the OriCell™ Mouse Embryonic Fibroblast Growth Medium to 37°C and add 9 mL of the medium into a 15 mL conical tube.
2. Remove one cryovial of OriCell™ KO-Certified 3R MEFs (Irradiated) from liquid nitrogen and quickly thaw at 37°C. For optimal post-thaw viability, be sure to limit the thawing process to 3 minutes.
3. Disinfect exterior walls of the cryovial with 70% ethanol and transfer the cells to a 15 mL centrifuge tube containing OriCell™ Mouse Embryonic Fibroblast Growth Medium.
4. Rinse the vial with 1 mL of medium and transfer the cell suspension into the centrifuge tube to reduce cell loss.
5. Gently mix the cell suspension by pipetting, and centrifuge at 250 x *g* for 5 minutes.
6. Carefully aspirate off as much of the supernatant as possible and add 3 mL of pre-warmed fresh OriCell™ Mouse Embryonic Fibroblast Growth Medium to gently resuspend the cells.
7. Seed the cells into 6-well plates (or other appropriate culture vessels) pre-coated with gelatin solution and add sufficient fresh OriCell™ Mouse Embryonic Fibroblast Growth Medium. Gently shake the culture plate to evenly distribute the cells. We recommend plating cells at 2.5-3.0×10^4 cells/cm^2 to obtain the appropriate seeding density.
8. Incubate at 37°C in a 5% CO_2 humidified incubator and replace the media with fresh media next day.