OriCell™ Strain 129 Mouse Embryonic Stem Cells (ESCs)

Product Name	OriCell™ Strain 129 Mouse Embryonic Stem Cells (ESCs)
Catalog No.	MUAES-01001
Product Size	1×10^6 Cells/ Vial
Passage Number	20
Storage	Liquid Nitrogen

PRODUCT OVERVIEW

Embryonic Stem Cells (ESCs) are pluripotent cells derived from the inner cell mass of blastocysts that retain the potential to differentiate into all 3 derivatives of the primary germ layers including ectoderm, endoderm, and mesoderm. Due to their unlimited self-renewal capacity and developmental plasticity, ESCs hold great promise in providing cell-based therapies in regenerative medicine and tissue replacement.

Cyagen OriCell™ Strain 129 Mouse ESCs are derived from the inner cell mass of strain 129 mouse blastocysts (at 3.5 dpc) and cultured on γ-ray irradiated mouse embryonic fibroblast feeder cells in OriCell™ Mouse ESC Growth Medium. They maintain diploid karyotype even after extended passages *in vitro* and express cell type-specific markers of ESCs. OriCell™ Strain 129 Mouse ESCs demonstrate effective in forming both embryoid bodies *in vitro* and teratomas in nude mice.

HIGHLIGHTED PRODUCT FEATURES

- Proven unlimited self-renewal capacity and pluripotency if maintained properly;
- Tested positive for ESC-specific markers including Oct4, SSEA-1 and Nanog (≥90%) and negative for multipluripotency-associated markers including SSEA-3 and SSEA-4 (≤5%);
- High post-cryostorage cell viability.

GENERAL HANDLING PRINCIPLES

- Use aseptic technique when handling this product to prevent microbial contamination;
- Cells should be stored at -80°C until use and maintained on MEF feeder cells. Seed cells at a density of 2.0-2.5×10^4 cells/cm^2 and maintain on Cyagen OriCell™ KO-certified 3R Mouse Embryonic Fibroblasts for optimal results;
- Passage cells every 2-3 days to obtain optimal cell culture confluency and maintain the cells as single colonies.

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. Thawing and Establishing OriCell™ Strain 129 Mouse Embryonic Stem Cells (mESCs)

Materials Needed

- OriCell™ Strain 129 Mouse Embryonic Stem Cells (Cat. No. MUAES-01001)
- OriCell™ Mouse Embryonic Stem Cell Growth Medium (Cat. No. MUXES-90011)
- 15 mL aseptic conical tubes
- 37°C water bath
- Tissue culture flasks or vessels of proper sizes

Procedure

1. Prior to plating, warm OriCell™ Mouse ESC Growth Medium and 1×PBS to 37°C. Add 9 mL of OriCell™ Mouse ESC Growth Medium into a 15 mL conical tube.
2. Remove one cryovial of OriCell™ Strain 129 Mouse ESCs from liquid nitrogen and quickly thaw in 37°C water bath. For optimal post-cryopreservation cell viability, limit thawing to no more than 3 minutes.
3. Disinfect exterior walls of the cryovial with 70% ethanol and transfer the cells to a 15 mL conical 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 the cell suspensions at 250 x *g* for 5 minutes.
6. Carefully aspirate off all supernatant and add 3 mL of fresh OriCell™ Mouse ESC Growth Medium (pre-warmed to 37°C) to re-suspend the cells.
7. Plate cells at the appropriate seeding density into your tissue culture flasks or vessels that contain sufficient amount of OriCell™ Mouse ESC Growth Medium. Gently rock the culture flask to evenly distribute the cells.
8. Incubate at 37°C in a 5% CO_2 humidified incubator and replace with fresh media next day.

II. Passaging and Maintaining OriCell™ Strain 129 mESCs on MEF Feeder Cells

Materials Needed

- OriCell™ Mouse Embryonic Stem Cell Growth Medium (Cat. No. MUXES-90011)
- Tissue culture vessels or flasks plated with MEFs of appropriate density
- 0.05% or 0.25% Trypsin-EDTA solution

Procedure

1. Prior to cell passage, warm OriCell™ Mouse ESC Growth Medium, 1×PBS and Trypsin-EDTA solution to 37°C.
2. Remove the tissue culture dish platedwith OriCell™ ICR Mouse Embryonic Fibroblasts (MEF) from tissue culture incubator and aspirate off all the spent media in an aseptic biological hood.
3. Rinse with appropriate amount of 1×PBS. Repeat rinse one or two times.
4. Supplement the MEF-plated culture vessel with OriCell™ Mouse ESC Growth Medium and return the MEFs to 5% CO_2 humidified incubator. Be sure to not disturb the monolayer of MEFs in culture.

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5. Carefully aspirate off spent medium from OriCell™ Strain 129 Mouse ESCs and rinse two or three times with 1×PBS (as in step 3-4).
6. Incubate OriCell™ Strain 129 Mouse ESCs with Trypsin-EDTA solution of proper concentration for 1-2 minutes (or longer) until cells dissociate. Add OriCell™ Mouse ESC Growth Medium to terminate the reaction and gently pipette up and down to make a single cell suspension.
7. Transfer the cells into a 15 mL conical tube and centrifuge at 250 x *g* for 5 minutes to pellet the cells.
8. Carefully remove as much supernatant as possible and gently mix pellets with 2 mL of OriCell™ Mouse ESC Growth Medium to resuspend the cells.
9. Plate mESCs into the tissue culture vessels plated with MEFs at an appropriate seeding density as directed below. Add sufficient OriCell™ Mouse Embryonic Stem Cell Growth Medium to cover the entire base of the culture dish and incubate at 37°C in a 5% CO_2 humidified incubator. Split cells and replace with fresh media every 2-3 days.

Note:

1. The ideal seeding density for OriCell™ Strain 129 Mouse ESCs should both allow for even distribution of cells over the tissue culture vessel surface and ensure the cells grow as single colonies. Differentiation may occur if cells are plated too densely or sparsely.
2. Beware to minimize the number of passage and time length in culture of OriCell™ Strain 129 Mouse ESCs. This will be beneficial to the maintenance of biological function of cells.

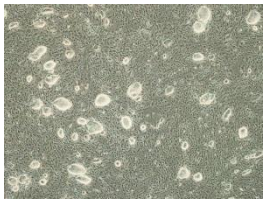

Fig.1 OriCell™ Stain 129 Mouse Embryonic Stem Cells at passage 21 are maintained on OriCell™ ICR Mouse Embryonic Fibroblasts (Irradiated) feeder cells and display healthy growth.

III. Inducing OriCell™ Strain 129 mESCs to Form Embryoid Bodies

Embryoid bodies (EBs), a type of multi-cellular three-dimensional aggregate structures, are formed when ESCs are cultured in suspension in the absence of anti-differentiation factors. Known as a hallmark of ESC differentiation, EBs recapitulate many aspects of multicellular-interactions. In literature, several methods are described to induce EB formation from ESCs. The protocol below provides one approach to establish an *in vitro* ESC differentiation system.

Materials Needed

- OriCell™ Embryoid Body (EB) Formation Medium (Cat. No. MUXES-90051)
- 100mm non-adherent tissue culture dish
- Regular high-binding tissue culture vessels

Procedure

1. Incubate OriCell™ Strain 129 Mouse ESCs with trypsin solution at 37°C for 1-2 min until cells are dissociated. Add an appropriate volume of Cyagen OriCell™ EB Formation Medium (e.g. 3 mL for each well of six-well plate) to terminate the reaction and gently pipette until cells dispense to form a single cell suspension.
2. Transfer the cell suspension into a 15 mL conical tube and centrifuge at 250 x *g* for 5 minutes.
3. Carefully aspirate as much supernatant as possible and gently mix with appropriate amount of Cyagen OriCell™ EB Formation Medium to resuspend the cells.
4. Plate the cell suspension in a tissue culture dish of appropriate size and incubate at 37°C 5% CO_2 incubator for 30-40 minutes to separate MEF adherent culture from OriCell™ Strain 129 Mouse ESC suspension culture.
5. Carefully collect the suspended OriCell™ Strain 129 Mouse ESCs and mix with OriCell™ EB Formation Medium to obtain a cellconcentration of approximately 5 x 10^5 cells/ml.
6. Plate 10 mL cell suspension in one 100 mm non-adherent culture dish and incubate at 37°C in a 5% CO_2 humidified incubator for 5 days to allow for EB formation. Replace with fresh media every other day.
7. Plate EB into adherent surface of gelatin coated tissue culture vessels containing Cyagen OriCell™ EB Formation Medium. Incubate at 37°C in a 5% CO_2 humidified incubator for 14 days. Replace with fresh media every other day.
8. Immunostain the cells with antibodies against endoderm, mesoderm and ectoderm-specific protein markers at day 14 to verify ESC differentiation and EB formation.

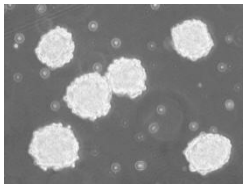

Fig.2 OriCell™ Strain 129 Mouse Embryonic Stem Cellsare induced to form embryoid bodies *in vitro* after incubation with EB formation medium in the absence of MEF feeders for 2 weeks.

IV. Cryopreservation of OriCell™ Strain 129 mESCs

Materials needed

- OriCell™ NCR Protein-Free Cryopreservation Medium

1. 24 hours prior to cryopreservation, replace cell culture media. Cells must be in the logarithmic growth phase at time of freezing.
2. Centrifuge the cells at 20°C, 250 x *g* for 3-5 minutes. Remove the supernatant and resuspend the cell pellet with OriCell™ NCR Protein-Free Cryopreservation Medium. For optimal post-cryostorage cell viability, dilute cell suspension to obtain a cell density of 10^5-10^6 cells/ml approximately.
3. Dispense aliquots of the cell suspension into labeled cryogenic storage vials and place the vials immediately in a -80°C freezer. After 24 hours, transfer the frozen vials to liquid nitrogen for long-term storage.