

Indirect Co-culture of Embryonic Stem Cells with Embryonic Fibroblasts

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A. Day 1

1. Coat T-75 flasks with 10 mL of 0.1% gelatin in DPBS and incubate for at least 30 minutes at 37°C.
2. Thaw PMEF vial (s) quickly in a 37°C water bath, transfer to 15 mL tube already containing 10 mL warm medium. Gently invert tube, and pellet cells at 4°C, ~1000 rpm for approximately 4–5 minutes.
3. Remove supernatant, resuspend cells, remove gelatin from plates/flasks, and aliquote feeder cell suspension per densities recommended below.
4. Remove excess gelatin from flask prior to seeding.
5. Seed flask with MEF feeder cell suspension: approximately 1.5×10^5 cells per mL MEF should result in 95% confluence within 24 hours.
6. Incubate at 37°C overnight.

B. Day 2

1. Coat T-75 flasks with 10 mL of 0.1% gelatin in DPBS and incubate for at least 30 minutes at 37°C.
2. Thaw PMEF vial (s) quickly in a 37°C water bath, transfer to 15 mL tube already containing 10 mL warm medium. Gently invert tube, and pellet cells at 4°C, ~1000 rpm for approximately 4–5 minutes.
3. Remove supernatant, resuspend cells, remove gelatin from plates/flasks, and aliquote feeder cell suspension per densities recommended below.
4. Remove excess gelatin from flask prior to seeding.
5. Seed flask with MEF feeder cell suspension: approximately 1.5×10^5 cells per mL MEF should result in 95% confluence within 24 hours.
6. Incubate at 37°C overnight.

C. Day 3

1. Feed ESC on MEF feeder layer with fresh ESC media.

D. Day 4-8

1. Feed ESC on MEF feeder layer with fresh ESC media or pass cells, at a 1:2 ratio, if required. (After thawing ESC, 2–3 passages are preferred before seeding onto a Millicell®-24 or Millicell® 96-well Cell Culture Insert Plate. Both cell types are lifted at once and passed on to a new T-75 containing inactivated MEF.) ESC colonies grown on Millicell®-96 Cell Culture Insert Plate 1.0 PET membrane, stained for alkaline phosphatase activity, after culturing via indirect co-culture with ESC in apical well, at a 200 cell per well seeding density, and MEF in the single-well feeder tray.

E. Day 9

1. Feed ESC on MEF feeder layer with fresh ESC media.
2. Coat Millicell®-24 or Millicell®-96 single-well feeder tray with approximately 5–10 mL of 25 µg/mL fibronectin in DPBS and incubate for 45 minutes at room temperature.
3. Remove excess fibronectin from single-well tray.
4. Thaw MEF using protocol from Day 1, section A.
5. Seed fibronectin coated single-well tray with MEF cell suspension: approximately 1.67×10^6 MEF cells per single-well tray will result in 95% confluence within 24 hours.
6. Cover with lid and incubate single-well tray at 37°C overnight.

F. Day 10

1. Lift ESC and MEF feeder cells from T-75 flasks:
2. Wash flasks 2X with 10 mL of pre-warmed DPBS (incubate 1–2 minutes per wash).
3. Remove DPBS and add 3 mL TrypLE and incubate at room temp for 2–3 minutes.
4. Monitor detachment of cells with an inverted microscope and add ESC media to inactivate TrypLE.
5. Mix well and wash flask wall to remove all cells from flask.
6. Separate ESC from MEF feeder cells:
7. Transfer ESC/MEF cell suspension to a new T-75 flask and incubate at 37°C for 45 minutes.
8. Remove non-adherent cells (ESC) and transfer to another new T-75 flask and incubate at 37°C for 45 minutes.
9. Remove non-adherent cells (ESC) again and seed cell culture filter plate wells with ESC suspension.
10. Seed apical wells of the cell culture plates with ESC. Seed approximately 200–500 cells per well in 100 µL ESC media for Millicell®-96 plates and approximately 1000–1500 cells per well in 400 µL ESC media for Millicell®-24 plates.
11. Remove media from the MEF seeded single-well trays and replace with approximately 28–32 mL ESC media.
12. Combine ESC seeded cell culture filter plates to MEF seeded single-well trays.
13. Incubate assembly at 37°C overnight.

G. Day 12 and Day 14

1. Feed ESC and MEF indirect co-culture with ESC media.

H. Day 16

1. Analyze alkaline phosphatase activity to demonstrate that ESC is undifferentiated with an alkaline phosphatase detection kit.

Note: This protocol is designed to grow undifferentiated embryonic stem cells in an indirect co culture with the fibroblast feeder layer. Although it is targeted for use with Millicell®-24 and Millicell®-96 plates, this protocol can be used with Millicell® single-well inserts as well.

Materials and Reagents

- Millicell®-24 Cell Culture Insert Plates — Millipore cat. nos. PSHT010R5, PSRP010R5
- Millicell®-96 Cell Culture Insert Plates — Millipore cat. no. PSRP004R5
- Primary mouse embryo fibroblasts (MEF) — Millipore cat. no. PMEFCFL
- 129/S6 Murine embryonic stem cells (ESC) — Millipore cat. no. SCR012

ESC Media:

- Knock out DMEM — Millipore cat. no. SLM-220-B
- 20% ES qualified Serum — Millipore cat. no. ES-009-B
- 1% Glutamax-1
- 1% PenStrep — Millipore cat. no. TMS-ABZ-C
- 1% Non Essential Amino Acids — Millipore cat. no. TMS-001-C
- 0.1% ESGRO® (LIF) — Millipore cat. no. ESG1106
- 0.1% 2-mercaptoethanol — Millipore cat. no. ES-007-E

MEF Media:

- DMEM — Millipore cat. no. SLM-022-B
- 10% Fetal Bovine Serum — Millipore cat. no. ES-009-B
- 1% Glutamax-1
- 1% PenStrep — Millipore cat. no. TMS-ABZ-C
- 1% Non Essential Amino Acids — Millipore cat. no. TMS-001-C
- Gelatin 2% Solution — Millipore cat. no. SF008
- Mitomycin C powder — Sigma cat. no. M4287
- DPBS — Millipore cat. no. BSS-1005-B
- TrypLE™ Select (1X), liquid — Invitrogen cat. no. 12563
- Tissue culture flasks and tubes
- Fibronectin Solution, 1mg/mL — Millipore cat. no. FC010

Other:

- Alkaline phosphatase detection kit — Millipore cat. no. SCR004

Materials

Product #	Description	Add to Cart
SCR004	Alkaline Phosphatase Detection Kit This Alkaline Phosphatase Detection Kit is a specific & sensitive tool for the phenotypic assessment of Embryonic Stem (ES) cell differentiation by the determination of AP activity.	pricing
SLM-220	EmbryoMax DMEM (1X), liquid, With 4,500mg/L Glucose, 2.25g/L Sodium Bicarb, without L-Glut and Sodium Pyruvate The EmbryoMax DMEM with 4,500mg/L Glucose, 2.25g/L Sodium Bicarb, without L-Glut & Sodium Pyruvate is available in a 500 mL format and may be used for routine mouse embryonic stem cell culture.	pricing
PMEFCFL	EmbryoMax Primary Mouse Embryonic Fibroblasts	pricing
SF008	ESGRO Complete Gelatin Solution	pricing
ESG1106	ESGRO Recombinant Mouse LIF Protein ESGRO Leukemia Inhibitory Factor (LIF) supplement for mouse ES cell culture. Each vial contains 10 ⁶ units/ml	pricing
FC010	Human Plasma Fibronectin Purified Protein This Human plasma fibronectin is a purified protein, used as an attachment factor suitable for cell propagation in vitro.	pricing
PIHA01250	Millicell Cell Culture Insert, 12 mm, HA mixed cellulose esters, 0.45 µm Mixed Cellulose Esters insert with pore size of 0.45 µm used in a 24-well plate for in vitro Toxicology Assays, Cell Attachment, cell culture, Cell Differentiation, Cell Surface Receptor Assays, Microbial Attachment Assays & Polarized Uptake Assays.	pricing
PIHA03050	Millicell Cell Culture Insert, 30 mm, HA mixed cellulose esters, 0.45 µm Mixed Cellulose Esters insert with pore size of 0.45 µm used in a 6-well plate for in vitro Toxicology Assays, Cell Attachment, cell culture, Cell Differentiation, Cell Surface Receptor Assays, Microbial Attachment Assays & Polarized Uptake Assays.	pricing
PICM01250	Millicell Cell Culture Insert, 12 mm, hydrophilic PTFE, 0.4 µm Hydrophilic PTFE cell culture insert with pore size of 0.4 µm used in a 24-well plate for Cell Attachment, Cell Culture, Cell Differentiation, Fluorescence & Low Protein Binding.	pricing
PICM0RG50	Millicell Cell Culture Insert, 30 mm, hydrophilic PTFE, 0.4 µm Hydrophilic PTFE cell culture insert with pore size of 0.4 µm used in a 6-well plate for Cell Attachment, Cell Culture, Cell Differentiation, Fluorescence & Low Protein Binding.	pricing
PICM03050	Millicell Cell Culture Insert, 30 mm, hydrophilic PTFE, 0.4 µm Hydrophilic PTFE cell culture insert with pore size of 0.4 µm used in a 6-well plate for Cell Attachment, Cell Culture, Cell Differentiation, Fluorescence & Low Protein Binding.	pricing
PIXP01250	Millicell Cell Culture Insert, 12 mm, polycarbonate, 12 µm Polycarbonate cell culture insert with pore size of 12.0 µm used in a 24-well plate for cell attachment, cell culture, cell differentiation, drug transport & permeability.	pricing
PIHP01250	Millicell Cell Culture Insert, 12 mm, polycarbonate, 0.4 µm Polycarbonate cell culture insert with pore size of 0.4 µm used in a 24-well plate for cell attachment, cell culture, cell differentiation, drug transport & permeability.	pricing
PIHP03050	Millicell Cell Culture Insert, 30 mm, polycarbonate, 0.4 µm Polycarbonate cell culture insert with pore size of 0.4 µm used in a 6-well plate for cell attachment, cell culture, cell differentiation, drug transport & permeability.	pricing
PITP01250	Millicell Cell Culture Insert, 12 mm, polycarbonate, 3.0 µm Polycarbonate cell culture insert with pore size of 3.0 µm used in a 24-well plate for cell attachment, cell culture, cell differentiation, drug transport & permeability.	pricing

Product #	Description	Add to Cart
PI8P01250	Millicell Cell Culture Insert, 12 mm, polycarbonate, 8.0 µm Polycarbonate cell culture insert with pore size of 8.0 µm used in a 24-well plate for cell attachment, cell culture, cell differentiation, drug transport & permeability.	pricing
PSHT010R5	Millicell-24 Cell Culture Insert Plate, polycarbonate, 0.4 µm Polycarbonate cell culture insert with pore size of 0.4 µm used in a 24-well plate for cell attachment, cell culture, cell differentiation, drug transport & permeability.	pricing
MCHT06H48	Millicell® Hanging Cell Culture Insert, PET 0.4 µm, 6-well, 48/pk Polyethylene Terephthalate hanging cell culture insert with pore size of 0.4 µm used in a 6-well plate for cell attachment, cell culture, cell differentiation & ICC.	pricing
MCHT12H48	Millicell® Hanging Cell Culture Insert, PET 0.4 µm, 12-well, 48/pk Polyethylene Terephthalate hanging cell culture insert with pore size of 0.4 µm used in a 12-well plate for cell attachment, cell culture, cell differentiation & ICC.	pricing
MCHT24H48	Millicell® Hanging Cell Culture Insert, PET 0.4 µm, 24-well, 48/pk Polyethylene Terephthalate hanging cell culture insert with pore size of 0.4 µm used in a 24-well plate for cell attachment, cell culture, cell differentiation & ICC.	pricing
MCRP24H48	Millicell® Hanging Cell Culture Insert, PET 1 µm, 24-well, 48/pk Polyethylene Terephthalate hanging cell culture insert with pore size of 1.0 µm used in a 24-well plate for cell attachment, cell culture, cell differentiation & ICC.	pricing
MCSP06H48	Millicell® Hanging Cell Culture Insert, PET 3 µm, 6-well, 48/pk Polyethylene Terephthalate hanging cell culture insert with pore size of 3 µm used in a 6-well plate for cell attachment, cell culture, cell differentiation & ICC.	pricing
MCSP12H48	Millicell® Hanging Cell Culture Insert, PET 3 µm, 12-well, 48/pk Polyethylene Terephthalate hanging cell culture insert with pore size of 3 µm used in a 12-well plate for cell attachment, cell culture, cell differentiation & ICC.	pricing
MCSP24H48	Millicell® Hanging Cell Culture Insert, PET 3 µm, 24-well, 48/pk Polyethylene Terephthalate hanging cell culture insert with pore size of 3 µm used in a 24-well plate for cell attachment, cell culture, cell differentiation & ICC.	pricing
MCMP06H48	Millicell® Hanging Cell Culture Insert, PET 5 µm, 6-well, 48/pk Polyethylene Terephthalate hanging cell culture insert with pore size of 5 µm used in a 6-well plate for cell attachment, cell culture, cell differentiation & ICC.	pricing
MCMP12H48	Millicell® Hanging Cell Culture Insert, PET 5 µm, 12-well, 48/pk Polyethylene Terephthalate hanging cell culture insert with pore size of 5 µm used in a 12-well plate for cell attachment, cell culture, cell differentiation & ICC.	pricing
MCMP24H48	Millicell® Hanging Cell Culture Insert, PET 5 µm, 24-well, 48/pk Polyethylene Terephthalate hanging cell culture insert with pore size of 5 µm used in a 24-well plate for cell attachment, cell culture, cell differentiation & ICC.	pricing
MCEP06H48	Millicell® Hanging Cell Culture Insert, PET 8 µm, 6-well, 48/pk Polyethylene Terephthalate hanging cell culture insert with pore size of 8.0 µm used in a 6-well plate for cell attachment, cell culture, cell differentiation & ICC.	pricing
MCEP12H48	Millicell® Hanging Cell Culture Insert, PET 8 µm, 12-well, 48/pk Polyethylene Terephthalate hanging cell culture insert with pore size of 8.0 µm used in a 12-well plate for cell attachment, cell culture, cell differentiation & ICC.	pricing
MCEP24H48	Millicell® Hanging Cell Culture Insert, PET 8 µm, 24-well, 48/pk Polyethylene Terephthalate hanging cell culture insert with pore size of 8.0 µm used in a 24-well plate for cell attachment, cell culture, cell differentiation & ICC.	pricing
MCRP06H48	Millicell Hanging Cell Culture Insert, PET 1 µm, 6-well, 48/pk Polyethylene Terephthalate hanging cell culture insert with pore size of 1.0 µm used in a 6-well plate for cell attachment, cell culture, cell differentiation & ICC.	pricing
MCRP12H48	Millicell Hanging Cell Culture Insert, PET 1 µm, 12-well, 48/pk Polyethylene Terephthalate hanging cell culture insert with pore size of 1.0 µm used in a 12-well plate for cell attachment, cell culture, cell differentiation & ICC.	pricing
M4287	Mitomycin C from <i>Streptomyces caespitosus</i> powder, BioReagent, suitable for cell culture	pricing
SCR012	PluriStem 129/S6 Murine ES cells The PluriStem 129S6 Murine ES cell line is derived from the widely used 129/S6/SvEv strain of mice (at 3.5 days p.c.) & is intended for injection into black or albino host blastocysts.	pricing