ES Cell Culture using ESGRO Medium Supplement

This protocol describes the *in vitro* culture of murine ES cells using the EmbryoMax range of ES cell qualified products with ESGRO supplement. Included are methods for ES cell culture with and without the inclusion of a PMEF feeder cell layer. The choice of method is dependent upon the ES cell line used, as certain ES cell lines require the use of feeder cells. When using PMEF cells, the maintenance of undifferentiated ES cells will be improved by the addition of ESGRO supplement to the cell culture medium.

Note: Optimized protocols for the culture of B6-White (Cat. No. SCR011) and129S6 (Cat. No. SCR012) ES cells can be found at www.millipore.com/chemicon.

ES Cell Culture without PMEF Feeder Cells

Materials & Reagents required:

- Centrifuge
- EmbryoMax DPBS (Cat. No. BSS-1006-A or BSS-1006-B)
- ES Cell Medium:
    - DMEM (Cat. No. SLM-220-B)
    - 15-20% Fetal Bovine Serum (Cat. No. ES-009-B or ES-011-B)
    - 1% Nucleosides,100x (Cat. No. ES-008-D)
    - 1% Penicillin-Streptomycin, 100x (Cat. No. TMS-AB2-C)
    - 1% Non-Essential Amino Acids, 100x (Cat. No. TMS-001-C)
    - 1% L-Glutamine Solution, 100x (Cat. No. TMS-002-C)
    - 1% 2-Mercaptoethanol, 100x (Cat. No. ES-007-E)
    - 1000 units/mL ESGRO mLIF Supplement (Cat. No. ESG1106 or ESG1107)
- Gelatin coated Tissue Culture Plates
- Incubator, 37 °C/5% CO_2
- Pipette
- 0.05% Trypsin-0.53 mM EDTA (Cat. No. SM-2002-C)

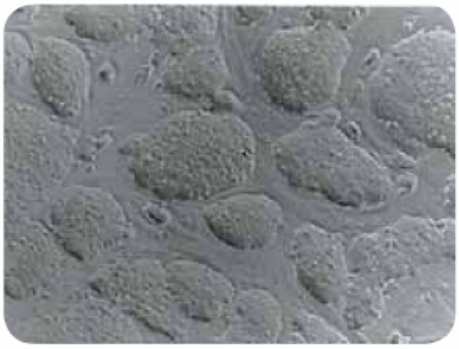
Procedure:

1. Thaw a vial containing $1x10^7$ ES cells into 4 mL of ES Cell Medium (containing ESGRO supplement at 1000 units/mL) and 4 mL of FBS. Centrifuge (3–5 minutes) and resuspend the cells in 10 mL of ES Cell Medium. Plate the ES cells onto the gelatinized plates at a density of 1–1.5 x 10^6 cells/25 cm^2 (~3 x 10^6 cells/100 mm plate). Incubate the plates at 37 °C with 5% CO_2. The cells appearance should resemble Image 5A (following page).
2. Examine the cells daily to determine if a change of media is required (indicated by a change of media color to yellow). After 2–3 days, ES cell cultures will become crowded with large colonies (see Image 5B). At this point, passage ES cells at a 1:2 ratio.
3. To passage ES cells, prepare two 100 mm gelatinized plates in advance. Remove ES Cell Medium, wash plates twice with DPBS, and add 1.2 mL of Trypsin. Incubate plates at 37 °C for 2 minutes, and then add 10 mL of ES cell medium. Pipette vigorously to break up the ES cell aggregates (avoid bubble formation).
4. Add 5 mL of the cell suspension to each gelatinized plate containing 5 mL of ES Cell Medium. Excess ES cells can be frozen at a concentration of 2–10 x 10^6 cells per vial for future use. Please note that ES cells should always be passaged the day before you intend to electroporate.

Image 5A

ES cells (R1) grown in the absence of PMEFs at the time of plating (10x)

Image 5B



ES cells (R1) grown in the absence of feeder cells after 4 days of culture (10x).

ES Cell Culture with PMEF Feeder Cells

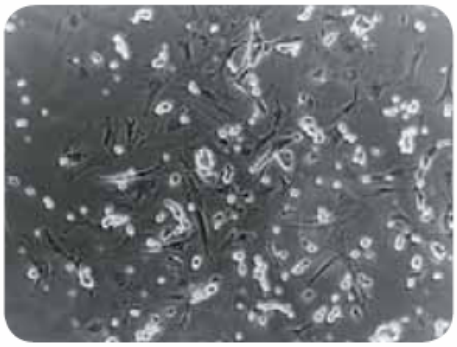
Materials & Reagents required:

- Centrifuge
- EmbryoMax DPBS (Cat. No. BSS-1006-A or BSS-1006-B)
- ES Cell Medium:
  - DMEM (Cat. No. SLM-220-B)
  - 15-20% Fetal Bovine Serum (Cat. No. ES-009-B or ES-011-B)
  - 1% Nucleosides,100x (Cat. No. ES-008-D)
  - 1% Penicillin-Streptomycin, 100x (Cat. No. TMS-AB2-C)
  - 1% Non-Essential Amino Acids, 100x (Cat. No. TMS-001-C)
  - 1% L-Glutamine Solution, 100x (Cat. No. TMS-002-C)
  - 1% 2-Mercaptoethanol, 100x (Cat. No. ES-007-E)
  - 1000 units/mL ESGRO mLIF Supplement (Cat. No. ESG1106 or ESG1107)
- Incubator, 37 °C/5% CO2
- Pipette
- PMEF Feeder cell coated plates
- 0.05% Trypsin-0.53mM EDTA (Cat. No. SM-2002-C)

Procedure:

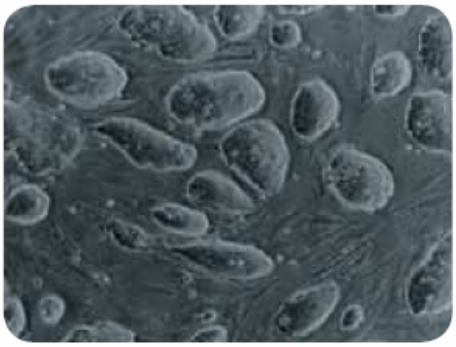
1. Thaw a vial containing $1x10^7$ ES cells into 4 mL of ES Cell Medium (containing ESGRO supplement at 1000 units/mL) and 4 mL of FBS. Centrifuge (3–5 minutes) and resuspend the cells in 10 mL of ES Cell Medium.
2. Remove the PMEF Feeder Cell Medium from a feeder plate prepared earlier (see Section 4), and seed the ES cells onto the PMEF coated plate at a density of 1–1.5 x 10^6 cells/25 cm2 (~3 x 10^6 cells/100 mm plate). Incubate the plates at 37 °C with 5% CO_2. The cells appearance should resemble Image 5C (below).
3. Examine the cells daily to determine if a change of media is required (indicated by a change of media color to yellow). After 2–3 days, the ES cell cultures will become crowded with large colonies (see Image 5D). At this point, passage the ES cells at a 1:2 ratio.
4. To passage ES cells, prepare two 100 mm plates containing PMEF cells as previously described (see Section 4). Remove the ES Cell Medium, wash plates twice with DPBS, and add 1.2 mL of Trypsin. Incubate at 37 °C for 2 minutes. Add 10 mL of ES Cell Medium and pipette vigorously to disperse the ES cell aggregates (avoid bubble formation).
5. Add 5 mL of the cell suspension to each of the PMEF cell plates containing 5 mL of ES Cell Medium. Excess ES cells can be frozen at a concentration of 2–10 x 10^6 cells per vial for future use. Please note that ES cells should always be passaged the day before you intend to electroporate.

Image 5C



ES cells (R1) grown in the presence of PMEFs at the time of plating (10x).

Image 5D



ES cells (R1) grown in the presence of feeder cells after 4 days of culture (10x).