# Plating PMEF Feeder Cells Protocol

EmbryoMax Primary Mouse Embryo Fibroblasts (PMEF) feeder cells are supplied as frozen vials containing 5–6 x 106 cells per vial at passage 3 (2–3 population doublings per passage).

It is recommended that PMEF feeder cells be plated one day prior to plating ES cells, which guarantees approximately 95% confluence of the PMEF cells. If ES cells are plated earlier than one day after PMEF plating, there may be some small gaps in the feeder layer. Although plating ES cells when gaps are present may not have any detrimental effects on the ES cells, it is not recommended.

**Materials & Reagents required:**

- 15 mL tubes
- Centrifuge
- EmbryoMax Cryopreserved PMEF Feeder Cells
- Gelatin coated Tissue Culture Plates or Flasks
- Incubator, 37°C/5% $CO_2$
- PMEF Feeder Cell Medium:
    - DMEM (Cat. No. SLM-220-B)
    - 10% Fetal Bovine Serum (Cat. No. ES-009-B or ES-011-B)
    - 1% Penicillin-Streptomycin, 100x (Cat. No. TMS-AB2-C)
    - 1% L-Glutamine Solution, 100x (Cat. No. TMS-002-C)
- Pipette
- Water Bath, 37°C

**Procedure:**

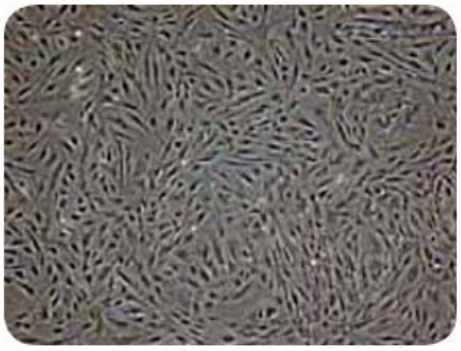
1. Prior to thawing PMEF feeder cells, coat plates/flasks with Gelatin solution
2. Thaw PMEF vial(s) quickly in a 37 °C water bath and transfer to a 15 mL tube (already containing 10 mL of warm PMEF Feeder Cell Medium). Gently invert the tube to distribute, and centrifuge at 300 xg for 4–5 minutes.
3. Remove supernatant and resuspend the cell pellet in warm PMEF Feeder Cell Medium. (see Table 4.1 for volumes.)

4. Remove the Gelatin solution from plates/flasks, and aliquot the PMEF feeder cell suspension at the densities recommended in Table 4.1 on the following page.
5. Incubate the PMEF Feeder cells at 37 °C with 5% CO2. Use images 4A, B and C as a guide for an estimate of correct PMEF density and appearance. Gelatinized plates may be used for 12–14 days.

Table 4.1: Recommended PMEF feeder cell suspension densities

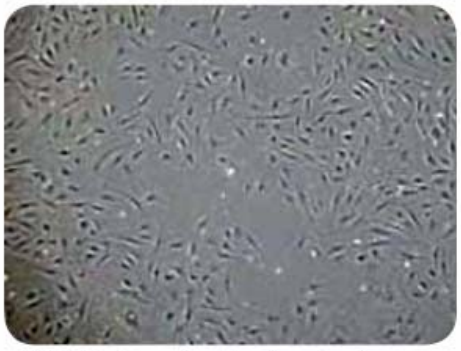
| Dish Size | Volume (mL)/ flask or well | Growth Area ($cm^2$) | No. of feeder cells/flask or well |
|---|---|---|---|
| 75 $cm^2$ flask | 12 | 75 | $3.75 \times 10^6$ |
| 25 $cm^2$ flask | 6 | 25 | $1.25 \times 10^6$ |
| 100 mm plate | 10 | 56 | $2.8 \times 10^6$ |
| 60 mm plate | 5 | 21 | $1.0 \times 10^6$ |
| 6-well plate | 4 | 9.5 | $4.75 \times 10^5$ |
| 12-well plate | 2 | 4 | $2.0 \times 10^5$ |
| 24-well plate | 1 | 2 | $1.0 \times 10^5$ |
| 96-well plate | 0.1 | 0.32 | $1.5 \times 10^4$ |

Image 4A



PMEF feeder cells at the correct density

Image 4B



PMEF feeder cells at too low density

Image 4C

PMEF feeder cells at too high density