



Applied StemCell

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## PROTOCOL

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### Mouse Embryonic Fibroblast (MEF) Cells (P3)

#### Cat. Numbers:

ASF-1013	ASF-1213	ASF-1223	ASF-1113
ASF-1015	ASF-1215	ASF-1225	ASF-1115

#### Protocol for Thawing MEF Cells

- Place a vial of frozen cells into a 37°C water bath immediately after removing from liquid nitrogen. Keep the cap out of the water to prevent contamination.
- As soon as the content of the vial starts thawing (~ 1 min), take the vial from the water bath and spray it with 70% Ethanol.
- Transfer the content to a 15 ml conical tube containing pre-warmed medium (10 ml per vial).
- Spin at 1000 rpm for 5 min, discard supernatant, and resuspend the cell pellet in growth medium.
- Plate cells on a gelatin-coated plate or flask at a density of around 30,000-50,000 cells/cm<sup>2</sup> (see **Table 1** for the recommended cell numbers for a variety of cell culture vessels). Optimal density should be determined by the researcher for the specific application.
- Place the cells in 37°C incubator and let settle for one day before use as feeder layer for Embryonic Stem (ES) or induced Pluripotent Stem (iPS) cell culture.

Dish Size	Surface Area	Working volume	MEF per dish / well
100 mm	55 cm <sup>2</sup>	11 - 16.5 ml	1.7 - 2.8 x 10 <sup>6</sup>
60 mm	21 cm <sup>2</sup>	4.2 - 6.3 ml	0.65 – 1.1 x 10 <sup>6</sup>
35 mm	9 cm <sup>2</sup>	1.8 - 2.7 ml	0.27 – 0.45 x 10 <sup>6</sup>
T25	25 cm <sup>2</sup>	5 – 7.5 ml	0.75 - 1.25 x 10 <sup>6</sup>
T75	75 cm <sup>2</sup>	15 – 22.5 ml	2.25 - 3.75 x 10 <sup>6</sup>
T175	175 cm <sup>2</sup>	35 – 52 ml	5.25 - 8.75 x 10 <sup>6</sup>
6-well	9.5 cm <sup>2</sup>	1.9 - 2.9 ml	0.29 – 0.48 x 10 <sup>6</sup>
12-well	3.8 cm <sup>2</sup>	0.8 - 1.2 ml	0.11 – 0.19 x 10 <sup>6</sup>
24-well	1.9 cm <sup>2</sup>	0.4 - 0.6 ml	57,000 – 95,000
48-well	0.95 cm <sup>2</sup>	0.2 - 0.3 ml	22,500 – 47,500
96-well	0.32 cm <sup>2</sup>	100 - 200 µl	9,600 – 16,000

**Table 1:** Approximate growth surface areas and recommended MEF cell number for cell culture. Numbers can vary between plastic ware from different suppliers.

Antibiotic	Suggested concentration	
	Mouse ES cells	Human ES cells
Neomycin	200 µg/ml	50-150 µg/ml
Puromycin	1 µg/ml	0.5 - 1 µg
Hygromycin	120 µg/ml	10 - 40 µg/ml
6-Thioguanine	1 - 10 µg/ml	1 - 5 µg/ml

**Table 2:** Suggested drug concentration for Mouse and Human ES cell culture. Always perform a dose-response curve (“kill-curve”) to determine the optimal concentration.

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