

PRX- BALB/c J

Derived from JAX Stock mouse ID 000651: BALB/cJ

These cells were derived and expanded on primary mouse embryonic fibroblasts (**MEFs**) in medium containing **1000U/ml LIF** and cultured at **5 % CO₂** in humidified air at 37°C.

- Replenish medium daily
- Passage every other day
- Trypsinize gently using 0.05% trypsin/EDTA (HyClone # SH30236)

DAY 1: Thaw one vial of ESC directly into a 25cm² flask containing a confluent layer of inactivated MEFs and 5mls of freshly prepared ESC medium.

Note: Thaw a vial quickly in a 37°C water bath by gently shaking it until all the material has thawed. Spray the vial with 70% ETOH and wipe with kimwipe. Aseptically transfer the contents of the vial into the prepared 25cm² flask, which has been equilibrated with ESC medium in the incubator for 1-2 hours prior to thawing the ESCs.

DAY 2: Examine the cells under the phase contrast scope at 4x and 10x. ESC colonies should be visible. Depending on the density of the colonies, either replace the medium and return the culture to the incubator OR passage the cells to a 10cm dish containing a confluent layer of inactivated MEFs.

DAY 3: Examine the cells under the scope. If the cells were not passaged to a 10cm dish on day 2, do so today. If they are already on the 10cm dish, depending on the size and density of the colonies, either replace the medium or trypsinize the culture and freeze $\frac{2}{3}$ to $\frac{1}{2}$ in 2 cryogenic vials for future experiments and passage the remaining $\frac{1}{3}$ to $\frac{1}{2}$ of the cells to a fresh 10cm dish for electroporation in 20-24 hours.

Final volume: 250 ml

Component	Amount	Final Conc.	Vendor and Catalogue #
KO-DMEM	192.5 ml	80%	Invitrogen #10829-018
FBS*	37.5 ml	15%	Thermo Fisher/ HyClone FBS* <i>ES Cell Qualified</i> #SH30070.03 E
KSR	12.5 ml	5%	Invitrogen # 10828-028
NEAA	2.5 ml	1x	HyClone # SH30038.01
L glutamine	2.5 ml	2mM	HyClone # SH30034.02
Pen/Strep	2.5 ml	1x	HyClone # SV30010
LIF	25ul	1000U/ml	Chemicon # ESG1107
2- ME	250 ul of 1000x Stock	0.1mM	Sigma # M 7522 (make 1000x stock: dilute, 35ul in 5mls of PBS)

Mix all ingredients in the top of a 2micron PES filter unit and filter. Store at 4°C. Discard any unused medium after 10 days.