

PRX- 129X1/SvJ ES Cells

Derived from JAX Stock mouse ID #000691

These cells were derived and expanded on primary mouse embryonic fibroblasts (**MEFs**) in medium containing **1000U/ml LIF** and cultured at **7.5% CO₂** in humidified air at 37°C.

- Exchange medium daily
- Passage every other day
- Trypsinize gently using 0.05% trypsin/EDTA

DAY 1: Thaw one vial of ESC directly into a 25cm² flask containing a confluent layer of inactivated MEFs and 5.5mls of freshly prepared ESC medium.

Note: Thaw a vial quickly in a 37°C water bath by gently shaking until all of the frozen 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 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 size and 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 on day 2, do so today. If they are already in the 10cm dish, depending on the size and density of the colonies, either replace the medium, further expand, freeze stock vials, initiate experiment.

It is advisable to freeze ½ to ⅓ of the cells in one or two vials for future experiments and passage the remaining cells to a fresh dish of feeders for electroporation on day 4.

PRX-129 ESC MEDIUM

Final volume: 500ml

Component	Amount	Final concentration	Vendor	Catalogue number
DMEM, high glucose no pyruvate	405ml	85%	HyClone	SH30081.02
FCS- ESC qualified**	75ml	15%	HyClone	SH30070.03E
NEAA (MEM) 100x	5ml	1x	HyClone	SH30238.01
L glutamine (200mM)	5ml	2mM	HyClone	SH30034.01
HEPES (1M)	5ml	10mM	HyClone	SH30237.01
Pen/strep	5ml	1x	HyClone	SV30010.01
B-Mercaptoethanol	3.5ul	0.1mM	Sigma	M-7522
ESGRO- mouse LIF	50ul	1000U/ml	Chemicon	ESG1107

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.