

Murine Embryonic Stem Cell Protocol: 129 ES Cells

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These are classic mouse embryonic stem cell (mESC) culture conditions. The ES cells are cultured in medium containing DMEM, FBS, LIF and co-cultured with primary mouse embryonic fibroblasts (MEFs). Pluripotent mESC have a very distinct morphology when cultured under these conditions; growing as tightly clustered colonies with smooth phase bright borders. Mouse ES cells grow quickly and require daily maintenance.

Cell culture conditions for 129 ES cells:

- 7.5% CO₂ in humidified air
- 37°C
- Replace the medium or Passage daily
- passage by using 0.05% Thermo Scientific HyClone Trypsin/EDTA (SH30236)

Day 1: Thaw one vial of ES cells directly into a 25 cm² flask containing a confluent layer of inactivated MEFs and 5 mLs of freshly prepared 129 ES cell medium.

Thaw vial in 37°C water bath by gently shaking the tube until all but a small sliver of frozen material remains. Spray with ETOH and aseptically transfer the contents to the flask with freshly prepared medium, equilibrated in the incubator for 1-2 hours.

Day 2: Examine the cells under a phase contrast microscope. ES cell colonies should be readily visible. Depending on the density of the colonies, either replace the ES cell medium and return to the incubator overnight or passage the cells to a T-75 flask, containing a confluent layer of inactivated MEFs (see SC

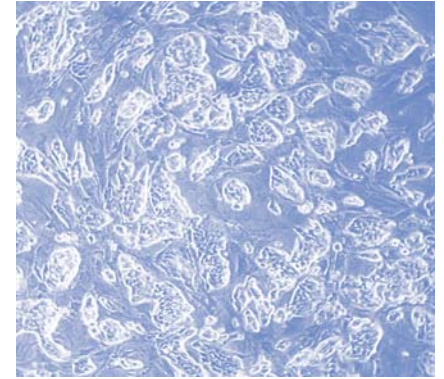


Photo courtesy of Primogenix, Inc.

Protocol Sheets 00002 and 00003) and 15 mLs of ES cell medium.

Day 3: If not already transferred to a T-75 flask, trypsinize the ES cells and transfer to a flask containing a confluent layer of inactivated MEFs and 15 mLs of 129 ES cell medium. If cells are passaged on day 2 replace the medium.

Day 4 or 5: Depending on the density and the size of the ES cell colonies:

Either replace the medium and allow the cells to proliferate another day or trypsinize the flask and freeze 50 % of the cells in three vials for later use and passage the remaining 50% of the cells to a new T-75 flask containing inactivated MEF feeders and 15 mLs of ES cell medium. Roughly 24 hours later the cells are ready for either electroporation, further expansion or experimentation.

Table 1: Preparation of 250 mL 129 ES cell medium

Brand	Amount for 200 mL	Product	Catalog Number
Thermo Scientific	205 mL	HyClone AdvanceSTEM™ DMEM4SC	SH30824
Thermo Scientific	35 mL	HyClone ES Screened FBS	SH30070(E)
Thermo Scientific	2.5 mL	HyClone AdvanceSTEM™ ES Qualified L-glutamine 200mM	SH30852
Thermo Scientific	2.5 mL	HyClone AdvanceSTEM™ ES Qualified Non-Essential Amino Acids (NEAA)100X	SH30853
Thermo Scientific	2.5 mL	Penicillin/Streptomycin Solution (optional)	SV30010
Thermo Scientific	2.5 mL	HyClone AdvanceSTEM™ ES Qualified HEPES (1M)	SH30851
Fisher	2.5 ul	2-ME	ICN19470580
Millipore	25 ul	ESGRO LIF	ESG1107
Mix all ingredients in the top of a 250 mL PES filter 0.22 µm unit and filter sterilize. Store at 4 C. Discard unused medium after 10 days.			

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