

# Mouse Embryonic Stem Cell Protocol: Primogenix Prx-129/X1 ES cells

Protocol  
SC 00006

*Adapted from Wesselschmidt, R. L. Primogenix, Inc.*

*For research use only*

## *Background*

These are classic mouse embryonic stem cell (mESC) culture conditions. The ES cells are cultured in medium containing DMEM, FBS, LIF (see Tables 1 and 2) and co-cultured with primary mouse embryonic fibroblasts (MEFs). Pluripotent mESC have 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.

## *Required Materials*

- Primogenix PRX-129/X1 ES cells (SV30098.01)
- Cell culture medium (see Table 1 and 2)
- Thermo Scientific HyClone Trypsin 0.05% (SH30236.01) or HyQTase (SV30030.01)
- Thermo Scientific Nalgene FastCap Bottle top filter (Fisher 09-740-68A, or equivalent)
- General cell culture supplies

## *Media Preparation*

Table 1: Preparation of Primogenix PRX-129/X1 ES cell medium with Thermo Scientific HyClone AdvanceSTEM™ DMEM4SC.

Product	Amount for 250 mL	Catalog Number
Thermo Scientific HyClone AdvanceSTEM DMEM4SC	197 mL	SH30824
Thermo Scientific HyClone ES Screened FBS	38 mL	SH30070(E)
Thermo Scientific HyClone AdvanceSTEM ES Qualified L-glutamine 200mM	7.5 mL	SH30852
Thermo Scientific HyClone AdvanceSTEM ES Qualified Non-Essential Amino Acids (NEAA) 100X	2.5 mL	SH30853
Thermo Scientific Penicillin/Streptomycin Solution (optional)	2.5 mL	SV30010
Thermo Scientific HyClone AdvanceSTEM ES Qualified HEPES (1M)	2.5 mL	SH30851
Fisher 2-ME	2.5 µL	ICN19470580
Millipore ESGRO LIF	25 µL	ESG1007
Mix all ingredients and sterile filter using a Thermo Scientific Nalgene FastCap Bottle top filter (Fisher 09-740-68A, or equivalent).		
Store at 4°C for up to 10 days.		

Table 2: Preparation of Primogenix PRX-129/X1 ES cell medium with Thermo Scientific HyClone AdvanceSTEM Low Osmo DMEM. The Low Osmo DMEM is recommended for optimal performance and is more inclusive than other classical media.

Product	Amount for 250 mL	Catalog Number
Thermo Scientific HyClone AdvanceSTEM Low Osmo DMEM	202 mL	SH30870
Thermo Scientific HyClone ES Screened FBS	38 mL	SH30070(E)
Thermo Scientific HyClone AdvanceSTEM ES Qualified L-glutamine 200mM	7.5 mL	SH30852
Thermo Scientific Penicillin/Streptomycin Solution (optional)	2.5 mL	SV30010
Millipore ESGRO LIF	25 µL	ESG1007
Mix all ingredients and sterile filter using a Thermo Scientific Nalgene FastCap Bottle top filter (Fisher 09-740-68A, or equivalent).		
Store at 4°C for up to 10 days.		

#### General Considerations

Cell culture conditions for Primogenix PRX-129/X1 ES cells:

1. 7.5% CO<sub>2</sub> in humidified air
2. 37°C
3. Replace the medium or passage daily
4. Passage by using HyQTase or Trypsin 0.05%

#### Thawing and Subculturing Cells

**Day 1:** Thaw one vial of Primogenix PRX-129/X1 ES cells (SV30098.01) directly into a 25 cm<sup>2</sup> flask containing a confluent layer of inactivated MEFs and 5 mLs of freshly prepared Primogenix PRX-129/X1 ES cell medium. Thaw vial in 37°C water bath by gently shaking the tube until all but a small sliver of the 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 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 Primogenix PRX-129/X1 ES cell medium. If cells are passaged on day 2 replace the medium.

**Day 4 or 5:** Depending on the density and 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 electroporation, further expansion or experimentation.

#### Related Protocols:

- SC Protocol 00001 - Mouse Embryonic Feeder Cell Protocol: Thawing Cryopreserved MEFs
- SC Protocol 00002 - Mouse Embryonic Feeder Cell Protocol: Subculturing MEFs
- SC Protocol 00003 - Mouse Embryonic Feeder Cell Protocol: Mitotic Inactivation of MEFs by Mitomycin C
- SC Protocol 00004 - Mouse Embryonic Feeder Cell Protocol: Cryopreservation of MEFs

#### References:

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