

# Mouse Embryonic Stem Cell Protocol: Primogenix B6N/X1 ES cells

Protocol  
SC 00005

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

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## *Background*

Pluripotent PRX-B6N ES cells have a distinct morphology when cultured under the following conditions, growing as tightly clustered colonies with smooth phase bright borders. Primogenix B6N ES cells grow quickly and require daily maintenance. When co-cultured with mouse embryonic fibroblasts (MEFs) in the medium described in tables 1 and 2, the cells are very robust.

## Required Materials

- Primogenix PRX-B6N ES cells (SV30109.01)
- 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-B6N ES cell medium with Thermo Scientific HyClone AdvanceSTEM™ DMEM4SC.

Product	Amount for 200 mL	Catalog Number
Thermo Scientific HyClone AdvanceSTEM IMDM4SC	145 mL	SH30822
Thermo Scientific HyClone ES Screened FBS	40 mL	SH30070(E)
Thermo Scientific HyClone AdvanceSTEM ES Qualified L-glutamine 200mM	2 mL	SH30852.01
Thermo Scientific HyClone AdvanceSTEM ES Qualified Non-Essential Amino Acids (NEAA) 100X	2 mL	SH30853.01
Thermo Scientific Penicillin/Streptomycin Solution (optional)	2 mL	SV30010
Fisher 2-ME	2 µL	ICN19470580
Millipore ESGRO LIF	20 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-B6N 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 200mL	Catalog Number
Thermo Scientific HyClone AdvanceSTEM Low Osmo DMEM	152 mL	SH30870
Thermo Scientific HyClone ES Screened FBS	40 mL	SH30070(E)
Thermo Scientific HyClone AdvanceSTEM ES Qualified L-glutamine 200mM	6 mL	SH30852
Thermo Scientific Penicillin/Streptomycin Solution (optional)	2 mL	SV30010
Millipore ESGRO LIF	20 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-B6N 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-B6N ES cells directly into a 25 cm<sup>2</sup> flask containing a confluent layer of inactivated MEFs and 5 mLs of freshly prepared Primogenix PRX-B6N 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-B6N 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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