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Cryopreservation of Neurons and ipSC.

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We use this protocol and it's working

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Abstract

This protocol describes cryopreservation and thawing methods for iPSC and derived neural cell types, including postmitotic neurons. It is based on standard protocols and approaches but recommends critical working reagents, timing, tips, and alternatives.

Guidelines

1. Other DMSO and non-DMSO cryopreservation media work as well. Homemade 10% DMSO with 50% FBS and 40%DMEM works only slightly, but significantly, less well for mouse or human PSC-derived motor neurons than millipore 2x FM; mFRSR from StemCell or FREEZEstem from Biolamina all work very nicely; StemCell banker is good too; vitrification in cryovials (see NYSCF Stem Cell or **Brivnalou protocols**; Scott Noggle adapted this from embryo culture in the early 2000s) is probably the highest viability approach; routinely preserves every single cell in the human blastocyst for reproduction. It also works on PSC clumps or single cells but is very labor-intensive.

2. Note: this approach has worked well for postmitotic motor, cortical, ventral forebrain, and dopaminergic neurons, neural progenitors, human embryonic/IPStem cells, mouse iPSC, sperm, microglia

Materials

1. EmbryoMax® 2X Freezing Medium for ES Cells, formulated with 20% DMSO & Fetal Bovine Serum.
Catalog No. ES-002-10F-Sigma Millipore.
2. Corning® CoolCell™ LX Cell Freezing Container.
Catalog No. CLS432001-Sigma Millipore.
3. 75% EtOH.
4. Cell culture media.

Safety warnings

1. ** IMPORTANT: Buffer osmotic changes are toxic, so a gradual change of buffer with mixing helps maintain viability.

2. IMPORTANT: Cryopreservatives are more dense than culture media and cells. Complete (but gentle!) mixing at each step must be ensured so your cells are not sitting at the bottom of the tube in 2x freezing media while the lighter culture media floats to the top. The same goes for thawing!

3. IMPORTANT: cold 1x freezing medium may be used; in that case, spin cells at 200-300G for 3 minutes after step 2 and resuspend gently in 1x freezing medium

4. *** IMPORTANT: The cryopreservation medium is very toxic. Limit the time the cells are exposed to it before they begin the controlled freeze.

5. **** Viability should be >50%, but more cells will die in the 1st 24 hours. Depending on cell type, overall viability can be 20-50%. Sometimes membranes are slightly leaky after thaw, so the trypan blue count is very high. Recount later. (AO/PI a better indicator of viability?)

Before start

1. Prelabel all tubes
2. Make fresh cell culture media

Cryopreservation

1 Prelabel all tubes and thaw freezing medium (Embryomax 2x freezing medium, for ES cells, Millipore); KEEP ON ICE. * ***See guideline #1 for information on other freezing media recommendations.***

2 Dissociate EBs/tissue/passage cells and resuspend cells at high density (5-20M cells/ml) in a plating medium (complete Neurobasal culture media (cNB), e.g., for neurons)

3 Add 1:1 volume of ice-cold 2x freezing media drop-wise, with swirling and pauses. It should take about 20-30seconds. ** ***See warning #1 for information on toxicity and how to improve cell viability at this step.***

4 Rapidly transfer tubes to a pre-cooled (4-degree) isopropanol-controlled freeze cylinder or other controlled rate freezer. *** ***See warning #4 for information on toxicity and how to improve cell viability at this step.***

5 Incubate freezing cylinder in -80 ON

6 Transfer FD aliquots to liquid nitrogen the next day (within the week. Viability decreases with time at -80)

Thawing

7 Thaw the FD aliquot in 37-degree water, gently swirling until only a few crystals remain.

8 Spray the FD tube with EtOH and rapidly transfer it to the hood.

9 Slowly add 1 volume (of freeze down, usually 1ml) medium (Neurobasal+B27-vitamin A), drop-wise w, swirling (15-30s). ***Note: Use the media cells are culture in.***

10 Transfer to a 15ml tube.

11 Rinse the cryovial with 1ml culture media

12 Add to 15ml tube

13 Slowly add 2 volume media, slowly with gentle mixing

14 Dilute to final ~10x FD volume, mix, and spin 3min 300_G

15 Count live cells***, and seed as fresh-dissociated cells.
a. A BSA cushion or Miltenyi Debris Removal solution suggested to remove debris.
b. Miltlenyii Dead Cell removal beads (antio-Annexin V) if low (<60%) viability is issuggested, follow manufacturer protocol. Note will lose ~100-200K cells. *** ***See warning #5 for information on viability at this step.***