Jun 14, 2024

Cryopreservation of stem cell derived ventral midbrain neural progenitors

DOI
dx.doi.org/10.17504/protocols.io.14egn6696l5d/v1

Tyra Fraser[1], Lachlan Thompson[1,2]

[1]Florey Institute of Neuroscience and Mental Health; [2]The University of Sydney

ASAP Collaborative Rese… Team Kirik

courtney.wright Wright
University of Sydney

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.14egn6696l5d/v1

Protocol Citation: Tyra Fraser, Lachlan Thompson 2024. Cryopreservation of stem cell derived ventral midbrain neural progenitors. **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egn6696l5d/v1

Protocol status: Working
We use this protocol and it's working

Created: May 31, 2024

Last Modified: June 14, 2024

Protocol Integer ID: 100999

Keywords: ASAPCRN, Cryopreservation, Neural progenitors, ventral midbrain, freezing, iPSC

Funders Acknowledgement:
Michael J Fox Foundation
Grant ID: ASAP-000497

Abstract

This protocol outlines the cryopreservation procedure for stem cell derived neural progenitors. It can be used for the cryopreservation and long-term storage of ventral midbrain dopamine neuron progenitors at Day 13 or Day 17 of differentiation in liquid nitrogen tanks.

Guidelines

All work is to be conducted in well sterilised laminar flow hoods designated for human iPSC work where possible to minimise contamination

Materials

Materials

- Mr.Frosty™ (isopropanol-based cell freezing container)Cryovials
- Labels
- P1000 pipette
- 15 mL falcon tubes
- Accutase
- Cryostor® CS10
- Rock inhibitor (Ri)
- NBB27

NBB27 Base media (For Terminal vmDA) 100ml	
DMEM/F12	47ml
NBM	47ml
B27 + VitA	2ml
N2	1ml
ITS-A	1ml
NEAA	1ml
GMAX	500ul
Pen strep	500ul

NBB27 base media for terminal vMDA

Safety warnings

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet) for each of the raw materials used

Before start

Day 17 (D17) VmDA and day 13 (D13) VmDA progenitors are used in this protocol

Experimental procedure

1d 0h 12m

1

9m

1. Take Mr. Frosty™ out of -80 °C freezer and keep at Room temperature .
2. Label the cryogenic vials with a label machine to include the following information: cell line, clone number, day of differentiation, freezing date and initials.
3. Wash cells with PBS -/-
4. Put 484 µL of Accutase per cm^2 of cells.
5. Incubate cells at 37 °C 00:04:00
6. Whilst cells are incubating place 5 mL of PBS -/- into a 15 mL Falcon tube.
7. Remove cells from incubator and pipette up and down twice. If cells come off in large clumps place them back into the incubator for 00:02:00. Repeat this step until cells form small clumps (~5cells). If cells form small clumps after the first 4-minute incubation move straight to step 8.
8. Place cells in the falcon tube prepared earlier (Step 6) with Ri 1:1000.
9. Spin cells 300 x g, 4°C, 00:03:00

2

3m

1. Aspirate supernatant and flick pellet twice.
2. Resuspend in 1 mL of NBB27+ Ri (1:1000).
3. Put 10 µL of cell mix into a small Eppendorf tube for cell counting.
4. Add 10 µL trypan blue to cells and mix well by pipetting up and down.
5. Transfer 10 µL of cells + Trypan blue mix in haemocytometer.
6. Count cells in each quadrant.
7. Calculate total number of cells.

Total cells = Average count of quadrants x Dilution factor x Volume (ml) x 10^4

8. Calculate volume required for desired freezing concentration. We recommend freezing no less than $1x10^6$ cells per vial.
9. Spin cells 300 x g, 4°C, 00:03:00

3

1d

1. Aspirate supernatant and flick pellet twice.
2. Resuspend in desired amount of Cryostor® CS10 for each cryovial (Calculated in Step 8 above).

***IMPORTANT**: Be fast to avoid cell death due to toxic Cryostor® CS10.*

3. Quickly aliquot 1 mL of cells + Cryostor® CS10 into cryovials.
4. Put the vials in Mr.Frosty and place in the -80°C freezer as fast as possible.

5. 24:00:00 after freezing, transport the cells on dry ice and store in Liquid Nitrogen for long-term storage.

IMPORTANT: *Do not* store cells in the -80 freezer for longer than 1 week.