Jun 15, 2024

Preparation of ventral midbrain cells for transplantation

DOI
dx.doi.org/10.17504/protocols.io.rm7vzjjkxlx1/v1

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Protocol Citation: Tyra Fraser, Lachlan Thompson 2024. Preparation of ventral midbrain cells for transplantation. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.rm7vzjjkxlx1/v1**

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

Created: May 31, 2024

Last Modified: June 15, 2024

Protocol Integer ID: 100998

Keywords: ASAPCRN, transplantation, ventral midbrain, iPSC, cell preparation, neurons

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

Abstract

This protocol outlines the preparation of iPSC derived ventral midbrain progenitors for xenotransplantation.

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

- PBS -/-
- Accutase
- P10, 20, 200, 1000
- 15ml falcon tube
- 3 small Eppendorf tubes
- Trypan blue
- NBB27 + ALL (add this fresh)
- Rocki (Ri)

	A	B	C
	NBB27 Base media (For Terminal vmDA) 100ml		+ALL (added as needed)
	DMEM/F12	47ml	BDNF (20ng/ml)
	NBM	47ml	GDNF (20ng/ml)
	B27 + VitA	2ml	TGFB3 (1ng/ml)
	N2	1ml	DAPT (10uM)
	ITS-A	1ml	AA (200uM)
	NEAA	1ml	dcAMP (0.25mM)
	GMAX	500ul	
	Pen strep	500ul	

Components of NBB27 base media

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

D19 iPSC derived ventral midbrain progenitors are used in this protocol

Experimental details

15m

1

8m

1. Wash wells with 1 x PBS -/-
2. Incubate cells in 484 µL of Accutase per cm^2 at 37 °C . **Note: D19 typically dissociate between 10-20mins.**
3. Prepare a 15 mL falcon tube for cells.
4. Check dissociation after 00:06:00 by using a p1000 to pipette up and down twice onto the
   cells. Observe if cells dislodge and are present in small clumps of 5-10 cells.
5. If **YES:** Collect all cells into a 15 mL tube. Wash wells with Accutase to ensure all cells are collected.
6. If **NO**: Incubate plate for intervals of 00:02:00 Pipette suspension up and down twice targeting
   the large clumps/sheets at each interval to promote dissociation. Repeat until cells are dissociated into small clumps when you can transfer to a 15 mL tube.

2

4m

1. Add Ri (1:1000)
2. Incubate the tube for 00:01:00 intervals until cells are broken into mostly single cells/small clumps (~5cells).
3. Cancel the reaction with PBS -/- at a 1:1 dilution to Accutase.
4. Spin cells (300 x g, 4°C, 00:03:00)

3

3m

1. Aspirate supernatant. Flick pellet twice.
2. Resuspend in NBB27 + All (~2ml per 48well) + Ri 1:1000.
3. Resuspend suspension and pipette 10 µL into a small Eppendorf tube for cell counting. Repeat
   for a second Eppendorf tube.
4. Take 2 tubes and add 10 µL trypan blue to cells in each tube.
5. Transfer 10 µL of mixed cells in haemocytometer.
6. Count cells in each quadrant.
7. Calculate total number of cells. Repeat this for tube 2 to ensure accurate cell counts.

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

8. Calculate the total volume needed to resuspend cells to a final density (typically between 100-150K/µl)and the amount of Ri 1:1000 required.
9. Spin cells (300 x g, 4°C, 00:03:00)

4

1. Aspirate supernatant.
2. Add half the required media on top of the cells gently (i.e. if you have 2 million cells total final volume is 20 µL to achieve 100 000 cells/µL so add 10 µL of media (NBB27 + All + Ri 1:1000) to pellet.
3. Using a P20 gently disturb the pellet in a circular motion, taking care not to damage cells by hitting the edge of the tube with the pipette tip.
4. Once mixed, take up the suspension once or twice and transfer to a small Eppendorf tube.
5. Measure the volume of cell suspension.
6. Add the appropriate volume of NBB27 + All + Ri 1:1000 required to reach the final volume.
7. Place cells on ice and transfer to surgical room immediately for transplantation.