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Recovery and preparation for transplantation of cryopreserved vmDA progenitors for transplantation.

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We use this protocol and it's working

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Abstract

This protocol outlines the recovery of cryopreserved vmDA progenitors. After recovery, this outlines the process to prepare these cells 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

General materials

- PBS -/-
- Accutase
- P10, 20, 200, 1000 plus tips
- 15ml falcon tubes
- 3 small Eppendorf tubes
- Trypan blue
- NBB27 + AII
- Rock inhibitor (Ri)

	A	B
	NBB27 Base media (For Terminal vmDA) 100ml	+ AII (add 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	

Required components for Base media NBB27

Troubleshooting

Safety warnings

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

Recovery of cells

1 Prepare NBB27 base media according to the materials table.

2 3m

1. Place 5 mL of NBB27 media + All + Ri 1:1000 in a 15 mL falcon tube.
2. Warm cryopreserved cells in hand or in a water bath until a small chunk of ice is remains
3. Remove thawed portion into the tube containing the 5 mL (prepared in as per Step 1)
4. Take 500 µL of media and use it to thaw the remaining chunk of ice.
5. Remove all media from cryopreserved tube and place into the falcon tube with media.
6. Spin cells(300 x g, 4°C, 00:03:00).

3 3m

1. Aspirate supernatant. Flick pellet twice.
2. Resuspend in 1 mL of NBB27 + All + Ri 1:1000.
3. Pipette 10 µL into a small Eppendorf tube for cell counting. Repeat for a second Eppendorf tube.
4. Take the 2 tubes and add 10 µL trypan blue to cells in each tube.
5. Place 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 an accurate cell count.

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.
9. Spin cells(300 x g, 4°C, 00:03:00)

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1. Label small Eppendorf tube with specific details about the transplant.
2. Aspirate supernatant
3. 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/uL, so add 10 µL of media
   (NBB27 + AII + Ri 1:1000) to pellet.
4. 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.
5. Once mixed, take up the suspension once or twice and transfer to a small Eppendorf tube.
6. Measure the volume of cell suspension.
7. Add the appropriate volume of NBB27 + AII + Ri 1:1000 required to reach the final volume.
8. Place cells on ice ready for use