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## Preparation of human iPSC-derived cortical neuronal progenitors for transplantation into the rodent brain

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**Protocol status:** Working

**We use this protocol and it's working**



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## Abstract

This protocol describes how we prepare human iPSC-derived cells, that have been differentiated into cortical neuronal progenitors, for transplantation into the brain of immunocompromised athymic mice. Neuronal progenitors mature within the mouse brain and are used to study the pathogenesis of Parkinson's disease.



## Materials

### Material input

- Adult (8-10 week) athymic mice (BALBc/Nu)
- IPSCs differentiated into cortical progenitors (D25-30 of differentiation depending on desired experimental outcomes), typically grown in 48 well plates. Full details of the cortical differentiation protocol: [dx.doi.org/10.17504/protocols.io.bu6znzf6](https://doi.org/10.17504/protocols.io.bu6znzf6).

### Equipment

- Microscope
- Microscope (brightfield with Phase contrast)
- Hemocytometer
- Pipettes (P20, P200, P1000)

### Consumables

- Falcon tubes
- PCR tubes
- pipette tips

### Key reagents

-  1X PBS (Phosphate-buffered saline )
-  ACCUTASE™ 100 mL **STEMCELL Technologies Inc. Catalog #7920**
-  Rock inhibitor Y-27632 dihydrochloride **Tocris Catalog #125410**
-  Trypan Blue 100 mL **STEMCELL Technologies Inc. Catalog #7050**

### Solutions

- Cortical Base Media (recipe [dx.doi.org/10.17504/protocols.io.bu6znzf6](https://doi.org/10.17504/protocols.io.bu6znzf6)): DMEM/F12, Neurobasal media, B27 supplement, N2 supplement, ITS-A, Glutamax, Pen/Strep,  $\beta$ -mercaptoethanol

## Safety warnings

 For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).



## Preparation of cell suspension

23m

1. Prepare a Falcon tube with  2 mL base cortical media plus Rock inhibitor (Ri, 1:1000 dilution)
2. Wash cells with  300  $\mu$ L PBS +/- (gently run PBS down the side of each well)
3. Incubate cells in  300  $\mu$ L Accutase (per well) at  37  $^{\circ}$ C to lift cells off wells in small clumps
  - Monitor the Accutase incubation after  00:10:00 ; tap the plate to dislodge cells and look for cells lifting in a sheet before proceeding
  - Triturate 5 times to break the cells into clumps
  - Incubate clumps for a further  00:05:00 at  37  $^{\circ}$ C
  - Triturate 10 times to break the cells into single cells and small clumps
4. Transfer cells from the wells into the 15ml Falcon tube containing media + Ri (Step 1)
5. Rinse with plate with a further  300  $\mu$ L of Accutase to ensure all cells are collected
6. Spin cells at  4  $^{\circ}$ C ,  1300 rpm for  00:04:00
7. Discard supernatant
8. Resuspend cell pellet in 1-2ml base cortical media and add Ri (1:1000)
9. Count cells
  - Take 2 x  10  $\mu$ L aliquot of cells in two separate tubesDilute cells 1:1 using  10  $\mu$ L of trypan blue
  - Count cells in a  10  $\mu$ L aliquot from each tube using a haemocytometer
  - Calculate total number of cells (Total cells = Average count of quadrants x Dilution factor x Volume (ml) x  $10^4$ )
10. Calculate the total volume needed to resuspend cells to a final density of 100 000/uL
11. Spin cells at  4  $^{\circ}$ C ,  1300 rpm for  00:04:00
12. Whilst cells are spinning, make up  1 mL media + Ri which will be used to resuspend the cells for implantation surgery
13. At end of the spin, collect tube and discard supernatant
14. Using a P20, add  5  $\mu$ L and resuspend pellet in base cortical media + Ri
15. Transfer resuspended pellet to PCR tube and precisely measure the volume



16. Add the remaining volume needed for cells to reach a density of 100 000 cells/ul as calculated in step 10.
17. Label the PCR tube containing the cells with details of the cell line and concentration
18. Store cells on ice and transport to animal surgical room.

## Transplantation

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  1. Athymic mice undergo stereotactic surgery to receive a unilateral graft of 100,000 cortical progenitors in  1  $\mu$ L volume. The surgical coordinates from Bregma are AP: +1, ML: +/-1.5, DV: -1.5

For full details of procedures for implantation of cell suspension can be found at <https://www.protocols.io/view/transplantation-of-fetal-midbrain-dopamine-progeni-261ge4jq7v47/v1>

## Protocol references

Cortical differentiation protocol: <https://dx.doi.org/10.17504/protocols.io.bu6znzf6>