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Version 1

Cortical Organoid Differentiation Protocol in 96-well Plates V.1

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Gustavo MorroneParfitt¹, Adriana Cunha¹, Charlie Arber², Selina Wray²

¹University College London; ²UCL, Queen Square Institute of Neurology



Adriana Cunha

University College London

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for cortical organoid generation in 96-well plates. Based on Pasca et al. 2015 (<https://doi.org/10.1038/nmeth.3415>) with some modifications.

Materials

iPSC media

StemFlex medium

Media for EB generation

StemFlex medium + 10 μ M Y-27632.

Media for organoid differentiation

Base media: DMEM/F12 + Neurobasal + N2 + B27 (- Retinoic Acid) + Glutamax + NEAA + Beta-mercaptoethanol.

Days 0-8: Base media + 2 μ M XAV939 + 10 μ M SB431542 + 100 nM LDN193189.

Days 9-24: Base media + 20 ng/ml FGFb + 20 ng/ml EGF.

Days 25-50: Base media + 20 ng/ml BDNF + Antibiotic-Antimycotic (1X).

Days 50+: Base media.

Days 70+: Base media (B27 + vitA)



Generation of Embryoid Bodies (Day -2)

2d 0h 8m

- 1 Only start the differentiation into organoids after passaging the iPSCs at least 3 times since thawing. The day you create the EBs is considered Day -2.
- 2 Make up media:  50 mL of StemFlex +  10 micromolar (μM) of Rock inhibitor (Y-27632).
- 3 Aspirate media from 2 wells of iPSCs.
- 4 Wash with  1.5 mL PBS per well.
- 5 Add  1 mL of accutase to each well.
- 6 Incubate at  37 °C and 5% CO₂ for  00:05:00 5m
- 7 Add  4 mL of media (as above) to a Falcon tube.
- 8 Take cells in accurate and add them to the Falcon tube with media.
- 9 Centrifuge at  300 x g for  00:03:00 3m
- 10 Aspirate supernatant and resuspend in  1 mL of media.
- 11 Count cells and add  100 μL of cell suspension in media to each well of a 96-well V-bottom ultra-low attachment plate to achieve 15,000 cells per well (150,000 per plate).
- 12 Incubate for  48:00:00 2d



Day 0

- 13 Make up Day 0-8 media (according to Materials section).
- 14 Remove as much spent media as possible without disrupting the EBs, wash with PBS and add  70 μL Day 0-8 media per well.

Days 1-8

- 15 Feed the EBs daily with the Day 0-8 media until Day 8 by removing and adding  70 μL Day 0-8 media per well.

Days 9-24

- 16 Make up Day 9-24 media (according to Materials section).
- 17 Feed EBs/organoids once every two days with the Day 9-24 media.

Days 25-50

- 18 Make up Day 25-50 media (according to Materials section).
- 19 Transfer organoids to ultra-low attachment 24-well plates and incubate them on a shaker. Alternatively, transfer to T75 flasks treated with ultra-low attachment solution.
- 20 Feed organoids twice a week with Day 25-50 media.

Days 50+

- 21 Make up Day 50+ media (according to Materials section).
- 22 Feed organoids twice a week with Day 50+ media.



Days 70+

- 23 Make up Day 70+ media (according to Materials section).
- 24 Feed organoids twice a week with Day 70+ media.