

Dec 03, 2025

Version 2

Cortical Organoid Differentiation Protocol in 96-well Plates V.2

DOI

<https://dx.doi.org/10.17504/protocols.io.eq2ly62regx9/v2>

Gustavo Morrone Parfitt¹, Adriana Cunha², Charlie Arber², Selina Wray²

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



Adriana Cunha

University College London

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.eq2ly62regx9/v2>

Protocol Citation: Gustavo Morrone Parfitt, Adriana Cunha, Charlie Arber, Selina Wray 2025. Cortical Organoid Differentiation Protocol in 96-well Plates. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.eq2ly62regx9/v2> Version created by **Adriana Cunha**

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working.



Created: April 28, 2025

Last Modified: December 03, 2025

Protocol Integer ID: 189060

Keywords: iPSC, Organoids, Brain Organoids, well plates protocol for cortical organoid generation, cortical organoid differentiation protocol, cortical organoid generation, well plates protocol, well plate

Funders Acknowledgements:

TransNAT

Grant ID: MR/X008029/1

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 medium

StemFlex medium

Medium for EB generation (Day -2)

StemFlex medium + 10 μ M Y-27632 (Rock inhibitor).

Medium for organoid differentiation

Base medium: DMEM/F12 + Neurobasal + N2 + B27 (without Vitamin A) + GlutaMAX + NEAA + 2-mercaptoethanol.

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

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

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

Days 50+: Base medium + Antibiotic-Antimycotic (1X).

Days 70+: Base medium (B27 containing Vitamin A) + Antibiotic-Antimycotic (1X).

Before start

Passage the iPSCs at least 2-3 times since thawing.



Generation of Embryoid Bodies (Day -2)

2d 0h 8m

- 1 The day you generate the embryoid bodies (EBs) is considered Day -2.
- 2 Make up Day -2 medium (according to Materials section).
- 3 Aspirate the spent medium 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 Day -2 medium to a Falcon tube.
- 8 Take cells in accutase and add them to the Falcon tube with medium.
- 9 Centrifuge at  300 x g for  00:03:00 3m
- 10 Aspirate supernatant and resuspend cells in  1 mL of Day -2 medium.
- 11 Count cells and seed 15,000 cells in  100 µL of Day -2 medium per well (i.e. 150,000 cells/ml) of a 96-well V-bottom ultra-low attachment plate (S-BIO).
- 12 Leave in incubator at  37 °C and 5% CO₂ for  48:00:00 . 2d



Day 0

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

Days 1-8

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

Days 9-24

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

Days 25-50

- 18 Make up Day 25-50 medium (according to Materials section).
- 19 If desired, transfer organoids to ultra-low attachment 24-well plates and incubate them on a shaker.
- 20 Feed organoids twice a week with Day 25-50 medium. If kept in 96-well plates, continue feeding organoids every two days.

Days 50+

- 21 Make up Day 50+ medium (according to Materials section).
- 22 Feed organoids twice a week with Day 50+ medium. If still in 96-well plates, continue feeding organoids every two days.



Days 70+

- 23 Make up Day 70+ medium (according to Materials section).
- 24 Feed organoids twice a week with Day 70+ medium. If still in 96-well plates, continue feeding organoids every two days.