Oct 24, 2025 Version 1

Cortical Organoid Protocol and Calcitriol treatment V.1

DOI
dx.doi.org/10.17504/protocols.io.kxygx4mb4l8j/v3

Katharina Meyer[1]

[1]Wyss Institute

Katharina Meyer
Wyss Institute

DOI: https://dx.doi.org/10.17504/protocols.io.kxygx4mb4l8j/v3

Protocol Citation: Katharina Meyer 2025. Cortical Organoid Protocol and Calcitriol treatment. **protocols.io https://dx.doi.org/10.17504/protocols.io.kxygx4mb4l8j/v3**

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

Created: July 10, 2025

Last Modified: October 24, 2025

Protocol Integer ID: 222232

Keywords: cortical organoid protocol, cerebral organoid, calcitriol treatment this protocol, calcitriol treatment, human brain development, microcephaly

Abstract

This protocol is based on the original protocol published by Lancaster et al. in 2013 (Lancaster, M., Renner, M., Martin, CA. *et al.* Cerebral organoids model human brain development and microcephaly. *Nature* **501**, 373–379 (2013). https://doi.org/10.1038/nature12517) with the following modifications

Materials

Media used during forebrain organoid culture:

Preparation of mTeSR1 Plus Medium with ROCK Inhibitor:
To prepare mTeSR1 Plus medium with ROCK inhibitor, combine the following components to achieve the specified final concentrations:

- mTeSR1: 100% (Thermo Fisher)
- ROCK Inhibitor (Y-27632, 10 mM stock): 50 µM final concentration (Stemcell Technologies)

Add the ROCK inhibitor to the mTeSR1 medium to reach a final concentration of 50 µM. Prepare and mix all components under sterile conditions. Use immediately or store as per manufacturer's recommendations.

Preparation of NIM (Neural Induction Medium):
To prepare NIM, combine the following components to achieve the indicated final concentrations:
DMEM-F12: 100%
N2 Supplement (100X): 1%
GlutaMAX (100X): 1X final concentration
MEM-NEAA (100X): 1X final concentration
Heparin (10 mg/ml): 1 µg/ml final concentration Add each component under sterile conditions. Mix thoroughly and, if necessary, filter sterilize before use.

Preparation of COD-Vit A / +Vit A Medium:
To prepare the COD-Vit A or +Vit A medium, combine the following components to achieve the specified final concentrations:

- DMEM-F12: 50%
- Neurobasal Medium: 50%
- N2 Supplement (100X): 0.5X final concentration
- B27 Supplement -Vit A: 1X final concentration
- Insulin: 1X final concentration
- GlutaMAX (100X): 1X final concentration
- MEM-NEAA (100X): 0.5X final concentration
- Penicillin/Streptomycin (100X): 1X final concentration
- 2-mercaptoethanol (2-BME) in DMEM-F12: add as a 1:100 dilution

First, prepare the 2-BME working solution by diluting it 1:100 in DMEM-F12, then add to the mixture. Combine all components under sterile conditions, mix thoroughly, and filter sterilize if necessary before use.

Troubleshooting

Generating embryoid bodies (EBs) from human iPSCs

1 Day 0: Add Y-27632 ROCK inhibitor (Selleckchem, #S1049) to iPSCs at 70-80% confluency, at a final concentration of 10µM. Allow cells to incubate at 37°C for at least 20 min

2 Add Accutase (Stem Cell Technologies) to facilitate cell detachment and to obtain a single cell solution

2.1 Incubate for 10-15 minutes and periodically check on cell detachment and gently tap plate to shake cells. Collect cells with appropriate amount of mTeSR1 Plus media supplemented with 10µM ROCK inhibitor

3 Transfer cells to a 15-ml or 50-ml Eppendorf tube.

4 Centrifuge cells at 1000g for 3 min.

5 Aspirate supernatant, and resuspend cell pellet in 1 ml mTesr1 media + 50 µM ROCK inhibitor.

6 Count cells using cell counter. Ideal cell count is at least 2 × 10^6 cells per ml.

7 Calculate and dilute cell suspension with mTesr1+50 uM ROCK to reach 9000 cells in a final volume of 150 ul per well.

8 Mix final cell solution by pipetting up and down. Pour cell solution into sterile reservoir.

9 Using multi-channel pipet, pipet 150 ul of cell solution into each well of a ULA 96-well plate.

10 Centrifuge 96-well plate(s) containing cells at 300 g for 3 min.

11 Check plate to ensure every well contains cells in a spherical shape.

12 Place in incubator and change media next day.

Day 1 and 2

13 Half media change using mTesr1 + 50 µM ROCK Inhibitor. Specifically, remove 75 µl media per well, and replace with 100 µl fresh media.

13.1 Ideally, use automated liquid handler (i.e. Integra Viaflow or Tecan Fluent).

14 Place back in 37°C incubator.

Day 3

15 Half media change using mTesr1 without ROCK. Specifically, remove 100 µl media per well, and replace with 100 µl fresh media.

16 EBs should be 350-600 µm in size. Also check for brightened smooth edges on EBs.

17 Place back in 37°C incubator.

Day 5: Neural induction of embryoid bodies

18 Carefully remove as much media as possible (full media change) without disturbing the EBs. Add 150 µl of Neural Induction Media (NIM). Avoid disturbing the EBs.

Day 7: Media Change

19 Half media change using NIM media (i.e. remove 75 µl old media, add 75 µl new media).

20 Observe translucent border for each EB.

Day 9: Embedding

21 EBs should be at least 800 um in size.

22 Prepare dimpled parafilm sheets ahead of time by using parafilm, 15 ml Falcon tube (bottom), and 200 µl tip box as a tray. Ensure all materials are sterile and further spraying liberally with 70% ethanol.

23 Thaw matrigel onto ice

24 Place the sterile parafilm sheet onto the 200 µl tip box tray.

25 Using wide-bore 200 µl tips or a cut pipet tip, gently aspirate each EB and transfer to a dimpled well onto the parafilm sheet.

26 Add 30 µl of cold matrigel onto each EB, on the dimpled parafilm. Immediately use a pipet tip to position the EB to the center of the Matrigel before polymerization starts.

27 Gently cover the embedded EBs (on parafilm sheet) with plate lid without crushing the domes. Place into 37°C incubator for 20 minutes. Continue with the rest of the EBs on the multichannel plane.

28 Pop each dimpled well of parafilm sheet upside down, and align it with a well plate (sterile ULA 6-well). Carefully flush the embedded EBs into the well using IDM-Vit A media.

28.1 Maximum 12 EBs per 6-well, and 5 ml media per well.

29 Place embedded EBs back in 37°C incubator. Observe for polarized neuroectoderm.

Day 11

30 Half media change by aspirating 2.5 ml old media from each well. Add 2.5 ml fresh IDM-Vit. A media.

31 Place embedded EBs back in 37°C incubator.

32 Repeat half media change every 2 days.

Day 16

33 Remove as much media as possible without aspirating embedded EBs. To do this, gently tilt the 6-well plate, allow EBs to fall to the bottom of the well, and aspirate from the sides or top.

34 Replace with IDM+Vit A media (final volume of 5 ml).

35 Change half media every 2 days. If media is yellow, perform full media change.

Day 18: Place EBs onto shaker (Shaking phase)

36 Full media change using IDM+Vit A.

37 Place embedded EBs onto shaker in 37°C incubator at 90 rpm.

38 Change half media every 2 days, and full media change every 3 days.

38.1 If media is yellow, perform full media change.

Day 46: Treating forebrain organoids with Calcitriol

39 Start drug treatment and dosing by adding calcitriol at a final concentration of 5 nM in IDM+Vit. A during media change.

40 Day 53: Collect forebrain organoids by washing once in PBS and then flash freezing in liquid nitrogen. Store at -80ºC until sample preparation