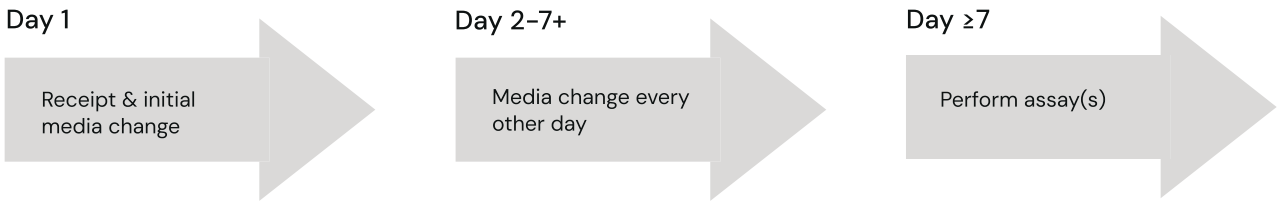
Protocol: CNS-3D Organoids 384-Well Plate

CNS-3D Organoid 384-Well Plates contain organoids comprised of induced pluripotent stem cell (iPSC)-derived cortical neurons and astrocytes. This document provides all necessary instructions for receipt and culture. Close adherence to the protocol enables success and full realization of the advantages of 28bio products.

Use: CNS-3D Organoid 384-Well Plates are intended for research use only. Recipients are responsible for the safe storage, handling, and use of CNS-3D Organoids. 28bio is not liable for any damages or injuries arising from the receipt and/or use of this product.

Workflow Overview



Media Preparation

IMPORTANT: PREPARE ALL MEDIA AND ESTABLISH LIQUID HANDLING SETTINGS PRIOR TO OPENING PLATE(S).

1. Prepare BrainPhys™ Complete Medium (STEMCELL™ Technologies) according to Table 1.
2. Store prepared media protected from light at 4°C for up to two weeks.
3. 28bio recommends adding Penicillin-Streptomycin (HyClone™ catalog # SV30010) at 1X concentration to the media at the time of preparation.
4. Prior to using the prepared media in cell culture, aliquot the amount needed and warm to room temperature.

Table 1: Preparation of BrainPhys™ Complete Medium

COMPONENT (SUPPLIER)	AMOUNT	FINAL CONCENTRATION	CATALOG #
BrainPhys Neuronal Medium and SM1 Kit (STEMCELL Technologies)	500 mL	1x for SM1 Supplement	05792
BDNF (STEMCELL Technologies)	10 µg	20 ng/mL	78005
GDNF (STEMCELL Technologies)	10 µg	20 ng/mL	78058
Penicillin-Streptomycin (HyClone) - *optional*	5 mL	1x	SV30010

Notes

- Automated liquid handlers for plate maintenance will require approximately 40 mL of media for the initial 3x media change and then 20 mL per media change thereafter (10 mL for priming + 10 mL for dispensing).
- For live cell fluorescence-based assays (e.g., FLIPR or Hamamatsu FDSS), we recommend BrainPhys Without Phenol Red (STEMCELL Technologies, Catalog #05791) plus NeuroCult™ SM1 Neuronal Supplement (STEMCELL Technologies, Catalog #05711) as the assay media in place of BrainPhys Neuronal Medium and SM1 Kit (Table1) to minimize signal interference.

Additional Equipment/Reagents

- Automated liquid handler (recommended: Appendix A)
- Multi-channel pipette
- Biosafety cabinet
- 70% Ethanol or Isopropanol
- 4°C refrigerator
- Corning Spheroid Microplate (Catalog number: 4516)

Receiving

5. Store plate(s) and lid(s) at room temperature until recovery. Cell culture recovery must occur the same day cells are received.
6. Transport package to a clean environment and remove CNS-3D Organoid 384-Well Plate(s) and accompanying sterile clear lid(s) from the external shipping box. Do not remove plate or lid wrapping materials.
    - For any issues with shipping, please contact customer service immediately: sales@28bio.com.

Removing External Plate Wrapping And Sealing Mat

7. Spray the plate(s) and lid(s) with 70% ethanol or isopropanol and transfer to a biosafety cabinet.
8. Remove the sterile clear lid from its packaging and set aside within reach.
9. Remove the external clear plastic wrapping from the plate while keeping it top side up (Figure 1).
10. Remove the top and bottom plastic covers. Place the sterile clear lid on the plate and over the mat.
11. Inspect the plate from the bottom, being careful not to tip it, to see if all spheroids are in the bottom of the wells. If not, centrifuge plate(s) for 2 minutes at 400xg. Repeat centrifugation step up to 2 additional times if needed to pull all spheroids down. Return the plate to the biosafety cabinet and remove the clear lid.
12. Hold the plate firmly from the base. Locate a corner of the sealing mat and **slowly** peel it off the plate revealing the wells. Take care not to reach over the open wells (Figure 2). If any media is seen outside the wells, use a vacuum aspirator to clean it up.

13. Place the clear lid on the plate and discard the sealing mat and packaging.
14. Repeat steps 8-13 for each additional plate(s) in the shipment.

Figure 1

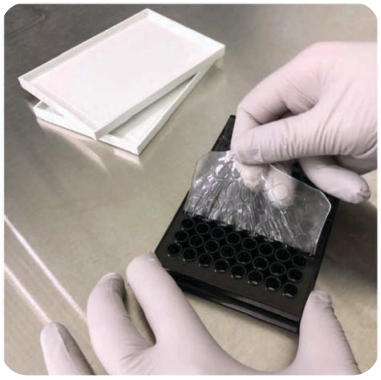


Figure 2

Initial Media Change

15. Automated liquid handler (suggested method):
    a. Establish the aspiration protocol for the CNS-3D Organoid plates per Appendix A.
    b. Initialize and prime dispensing cassette and tubing with 70% isopropanol (>50mL) followed by water (>50mL) and finally media (>10mL).
    c. Aspirate according to liquid handler specifications for your equipment, leaving 25 µL of media in each well.

       Note: Leaving less than 25 µL in each well may damage or aspirate the spheroid.
    d. Add 25 µL of prepared BrainPhys Complete Medium (Table 1).

       Note: With each media addition, wait a minimum of 30 seconds for spheroids to settle to the well bottom before proceeding to the next step.
    e. Aspirate 25 µL and add 25 µL prepared BrainPhys Complete Medium two additional times for a total of 3 media changes. The final working volume should be 50 µL per well.

OR

15. Multi-Channel Pipette:
    a. Add approximately 25 µL of prepared BrainPhys Complete Medium (Table 1) to each well, ensuring the meniscus is slightly lower than the top of the well and media is not overflowing into adjacent wells.

       Note: Avoid lowering the pipette too deep in the well to prevent touching and damaging the spheroids.

       Note: Ensure all well volumes are about the same height on average. May need to add additional media to some wells.

b. Wait 30 seconds for spheroids to settle to the bottom after each media addition.

c. Aspirate 75 µL of media, leaving about 25 µL left in the well. Then add 25 µL of prepared BrainPhys Complete Medium.

Note: : When aspirating with a multi-channel pipette, place pipette tip part way into the well at a 45° angle and aspirate slowly to avoid aspirating or damaging the spheroid.

d. Aspirate 25 µL and add 25 µL of prepared BrainPhys™ Complete Medium one additional time for a total of 3 media changes. The final working volume will be approximately 50 µL per well.

16. Repeat initial media change for each plate in the shipment.

17. Transfer the plate(s) to a cell culture incubator set to 37°C, 5% CO_2, and 95% to 98% humidity immediately after the final media change. Incubate for 2 days until the next media change.

Subsequent Media Changes

18. Culture plates for a minimum of one-week post shipment to allow for full cell recovery

a. **Example 1**: Shipment received and plates recovered on a Tuesday; media changes performed on Wednesday, Friday, and Monday; assay performed Tuesday

b. **Example 2**: Shipment received and plates recovered on a Wednesday; media changes performed Friday and Monday; assay performed Wednesday

19. Warm an aliquot of prepared BrainPhys Complete Medium to room temperature prior to each media change.

20. Perform half media changes every other day by aspirating 25 µL of old media and adding 25 µL of prepared BrainPhys Complete Medium (Table 1) to each well.

Note: Friday media replacement is adequate to carry through the weekend until Monday.

Perform Assay

21. After a minimum of one week in culture, the 28bio CNS-3D Organoid 384-Well Plate(s) are ready for experimentation.

Appendix A: Automated Liquid Handler Setup Information

Automated Liquid Handler (Recommended Method)

A1. Establish the aspiration protocol before changing media for CNS-3D Organoids.

A2. Ensure the automated system has the correct plate dimensions for the Corning Spheroid Microplate (Catalog Number 4516, Figure 3 below).

A3. Fill an empty Corning Spheroid Microplate with a liquid, such as water, and set the aspiration parameters to leave 25 µL per well. A slower aspiration is preferred for greater accuracy.

A4. Set the dispensing parameters so that the tip(s) has a low clearance from the top of the well/plate and is positioned over the center of the well. Use a low/slow flow rate that avoids turbulence and dispense 25 µL.

A5. Test the protocol and ensure 25 µL of fluid is aspirated then dispensed into each well.

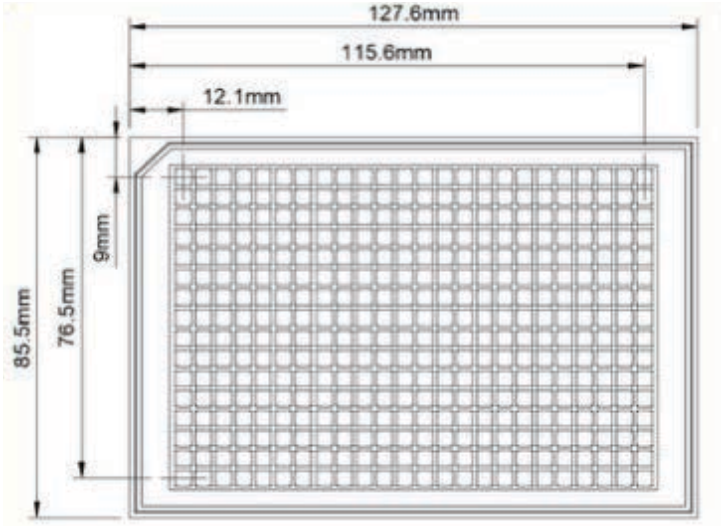


Figure 3. Corning Spheroid Microplate Measurements (Catalog Number 4516). Overhead view showing relevant width and length dimensions.

Key Dimensions for Corning Spheroid Microplate 4516:

- Total plate height = 14.20 mm
- Distance from bottom of plate to bottom of well = 1.81 mm
- Well bottom thickness = 0.09 mm
- Well depth = 12.30 mm