

CNS-3D Inflammatory Organoids: Receipt and Recovery Protocol

Purpose and use

[CNS-3D Inflammatory Organoids](#) contain induced pluripotent stem cell-derived cortical neurons, astrocytes, and microglia. This protocol provides instructions for receipt and ongoing culture in 96-well and 384-well plate formats.

Use: For research use only. Recipients are responsible for safe storage, handling, and use of CNS-3D Inflammatory Organoids. 28bio is not liable for damages or injuries arising from receipt or use of this product.

Required materials

Component
Automated liquid handler (recommended; see Appendix A)
Multi-channel pipette
Biosafety cabinet
70% ethanol or isopropanol
4°C refrigerator
Corning Spheroid Microplate (96-well #4520 or 384-well #4516)

Media preparation

IMPORTANT: Prepare medium and establish liquid-handler settings before beginning the assay.

1. Prepare BrainPhys Phenol Red Free Inflammatory Organoid Medium according to the table below.
2. Store prepared medium protected from light at 4°C for up to two weeks.
3. Before use, aliquot the required amount and allow it to warm to room temperature.

Component	Supplier	Catalog #	Amount	Stock Conc.	Final Conc.
BrainPhys Without Phenol Red	STEMCELL	05791	500 mL	N/A	1x
SM1	STEMCELL	05711	10 mL	N/A	1x
BDNF	STEMCELL	78005	10 µg	100 µg/mL	20 ng/mL
GDNF	STEMCELL	78058	10 µg	100 µg/mL	20 ng/mL
Penicillin-Streptomycin (optional)	HyClone	SV30010	5 mL	N/A	1x
Recombinant Human IL-34 Protein	R&D Systems	5265-IL-010/CF	10 µg	100 µg/mL	20 ng/mL

NOTE: Automated liquid handling requires approximately 20 mL of prepared medium per plate for each media change: approximately 10 mL for priming and 10 mL for dispensing.

NOTE: The phenol red free BrainPhys formulation is recommended to minimize signal interference during live-cell fluorescence-based assays, including calcium imaging.

Receive and inspect the plates

For shipping issues, contact 28bio immediately at support@28bio.com.

1. Using sterile gloves, carefully remove the plate(s) from the CellBox Shipper.
2. Return the plastic plate separators to the CellBox Shipper and discard the foam inserts. A CellBox representative will coordinate pickup of the unit.
3. Without tipping the plate, inspect it from below to confirm all organoids are at the bottom of their wells.
4. If any organoids are not at the bottom of the wells, centrifuge the plate for 2 minutes at 400 × g.
5. If needed, repeat centrifugation up to two additional times, inspecting the plate after each cycle.
6. Return the inspected plate to the incubator and repeat inspection for each additional plate.

Media changes

1. Perform a half-medium change upon plate receipt using the selected method and plate-format parameters.
2. Immediately after each media change, transfer the plate to a cell culture incubator set to 37°C, 5% CO₂, and 95–98% humidity.
3. Perform half-medium changes every other day using the selected method and plate-format parameters.

NOTE: Weekend scheduling: A medium change performed on Friday is sufficient until the next change on Monday; resume the every-other-day schedule thereafter.

NOTE: If performing multiple media changes at a time, wait a minimum of 30 seconds for organoids to settle to the well bottom before proceeding to the next media change.

Method A: Automated liquid handler (Recommended)

Action	96-Well Plate	384-Well Plate
Configure equipment	Use the 96-well liquid handler profile in the appendix.	Use the 384-well liquid handler profile in the appendix.
Prime dispensing path	Prime with >50 mL 70% isopropanol, >50 mL water, then >10 mL prepared medium.	Prime with >50 mL 70% isopropanol, >50 mL water, then >10 mL prepared medium.
Aspirate	Remove 100 µL; leave 100 µL in each well.	Remove 25 µL; leave 25 µL in each well.
Protect organoids	Do not reduce a well below 100 µL.	Do not reduce a well below 25 µL.
Dispense	Add 100 µL prepared medium.	Add 25 µL prepared medium.
Final Volume	200 µL per well.	50 µL per well.

Method B: Multi-channel pipette

Action	96-Well Plate	384-Well Plate
Aspirate	Remove 100 µL; leave 100 µL in each well.	Remove 25 µL; leave 25 µL in each well.
Protect organoids	Hold tips partway into wells at 45° and aspirate slowly.	Hold tips partway into wells at 45° and aspirate slowly.
Dispense	Add 100 µL prepared medium.	Add 25 µL prepared medium.
Final Volume	200 µL per well.	50 µL per well.

Assay

1. After 1 day of recovery, CNS-3D Inflammatory Organoids are ready for experimentation.

NOTE: Repeated medium exchanges within a single handling step can deplete microglia from CNS-3D Inflammatory Organoids. Standard culture handling, including half-medium changes every other day throughout the culture period, does not affect microglial content. Design workflows so that each handling step involves a single medium exchange. Assays requiring complete compound removal or repeated sequential washing within one step are not recommended for this model.

Appendix A: Automated liquid handler setup and plate specifications

Establish and verify the aspiration and dispensing protocol before changing medium in CNS-3D Inflammatory Organoid plates.

96-Well liquid handler profile

1. Ensure the automated system has the correct plate dimensions for the Corning® Spheroid Microplate (Catalog Number 4520, Figure 1 below).
2. Fill an empty Corning Spheroid Microplate with liquid, such as water.
3. Set the aspiration parameters to leave 100 μ L per well, with a low aspiration rate for greater accuracy.
4. Set the dispensing parameters so the tips have a low clearance from the top of the plate and are positioned over the well center. Use a low dispensing rate to avoid turbulence and dispense 100 μ L.
5. Test the protocol and confirm 100 μ L of fluid is aspirated from and then dispensed into each well.

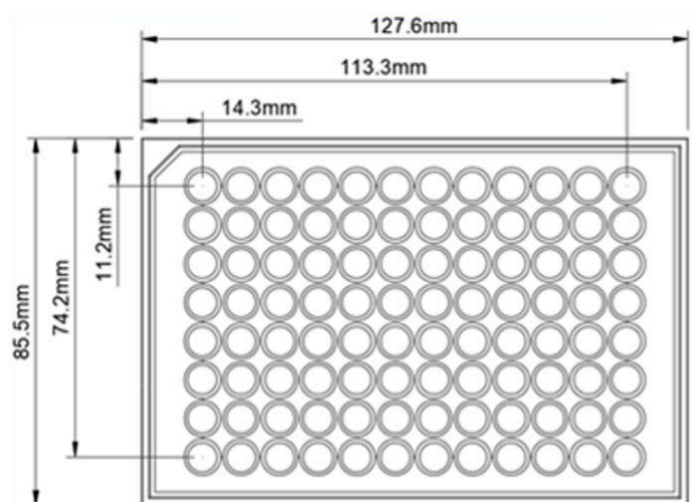


Figure 1. Corning spheroid microplate 4520 overhead dimensions

Key Dimensions for Corning Spheroid Microplate 4520 (96-well):

Specification	Value
Total plate height	14.20 mm
Distance from plate bottom to well bottom	1.86 mm
Well bottom thickness	0.09 mm
Well depth	12.10 mm

384-Well liquid handler profile

1. Ensure the automated system has the correct plate dimensions for the Corning® Spheroid Microplate (Catalog Number 4516, Figure 2 below).
2. Fill an empty Corning Spheroid Microplate with liquid, such as water.
3. Set the aspiration parameters to leave 25 μ L per well, with a low aspiration rate for greater accuracy.
4. Set the dispensing parameters so the tips have a low clearance from the top of the plate and are positioned over the well center. Use a low dispensing rate to avoid turbulence and dispense 25 μ L.
5. Test the protocol and confirm 25 μ L of fluid is aspirated from and then dispensed into each well.

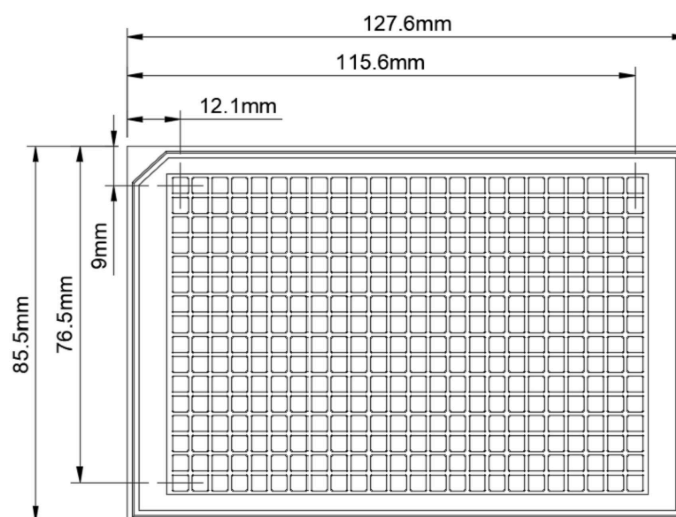


Figure 2. Corning spheroid microplate 4516 overhead dimensions

Key Dimensions for Corning Spheroid Microplate 4516 (384-well):

Specification	Value
Total plate height	14.20 mm
Distance from plate bottom to well bottom	1.81 mm
Well bottom thickness	0.09 mm
Well depth	12.30 mm

Product support

For product or shipping support, contact 28bio at support@28bio.com.