

Instructions for Use: StemoniX® microBrain® 3D Assay Ready 384-Well Plate

StemoniX® microBrain® 3D Assay Ready 384-well plates (BSARX-AA-0384) contain 3D spheroids of induced pluripotent stem cell (iPSC)-derived cortical neurons and astrocytes. This document provides all the necessary instructions for receipt and culture. Close adherence to the protocol enables success and full realization of the advantages of microBrain 3D assay ready products.

Use: StemoniX microBrain 3D Assay Ready 384-Well Plate(s) are intended for research use only. Recipients are responsible for the safe storage, handling, and use of StemoniX microBrain 3D Assay Ready 384-Well Plates. StemoniX 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 media according the Table 1.
2. Store prepared media protected from light at 4°C for up to one week.
3. StemoniX 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 you will need and warm to room temperature.

Table 1: Preparation of StemoniX NeuralX™ Medium¹

| Component | Basal Media Kits | |
|------------------------------------|---|---|
| | 250 mL Basal Media CAT # NXCNM-AA-0250 | 500 mL Basal Media CAT # NXCNM-AA-0500 |
| NeuralX Basal Media | Part # 500005-250 250 mL | Part # 500005-500 500 mL |
| NeuralX Basal Supplement | Part # 500011-005 5 mL | Part # 500011-010 10 mL |
| NeuralX Cortical Neuron Supplement | Part # 500012-010 250 µL | Part # 500012-020 500 µL |

¹See Appendix B if opting for BrainPhys based media

Additional Equipment/Reagents:

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

Receiving

5. Store plate(s) at room temperature until recovery. **Cell culture recovery should occur the same day cells are received.**
6. Transport package to a clean environment and remove StemoniX microBrain 3D Assay Ready 384-Well Plate(s) from the external shipping box. **Do not remove plate wrapping materials.**

Note: Minor leakage is acceptable if some volume of media is contained within each well. Vacuum aspiration can be used to clean any media leakage.

- a. For any issues with shipping, please contact customer service immediately at 1-855-783-6669 ext. 3, or customerservice@stemonix.com.

Centrifugation

7. Transfer the plate(s) to the cell culture lab and centrifuge plate(s) for 2 minutes at 400xg without removing plate wrapping.
8. Ensure the spheroids have settled to the bottom of the wells by inspecting the plate from below while keeping the plate upright.
 - a. Repeat centrifugation step up to 2 additional times.

Removing External Plate Wrapping and Sealing Mat

9. Spray the plate(s) with 70% ethanol or isopropanol and transfer to a biosafety cabinet.
10. Remove and discard the external wrapping while keeping the plate top side up (Figure 1).
11. Remove the lid and hold the plate firmly from the base. Locate a corner of the sealing mat and peel **slowly** in a diagonal direction across the plate (Figure 2).
12. Replace the lid on the plate and discard the sealing mat.

13. Repeat steps 10-12 for each additional plate in the shipment.

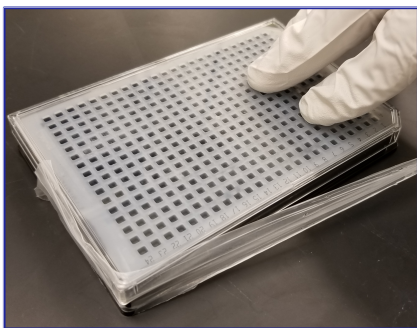


Figure 1

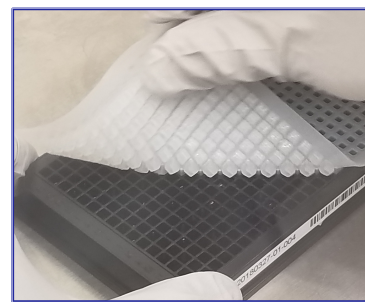


Figure 2

Initial Media Change

14. **Automated liquid handler** (suggested method):

- Establish the aspiration protocol for the microBrain Assay Ready plates per Appendix A.
- Aspirate according to liquid handler specifications for your equipment, leaving 25 μ L of media in each well.
Note: Leaving less than 25 μ L in each well may damage or aspirate the spheroid.
- Add 25 μ L of prepared NeuralX 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.
- Aspirate 25 μ L and add 25 μ L prepared NeuralX Medium two additional times for a total of 3 media changes. The final working volume should be 50 μ L per well.

OR

14. **Multi-Channel Pipette:**

- Add approximately 35 μ L of prepared NeuralX 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.
- Wait 30 seconds for spheroids to settle to the bottom after each media addition.
- Aspirate 75 μ L of media, leaving about 25 μ L left in the well. Then add 25 μ L of prepared NeuralX 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.
- Aspirate 25 μ L and add 25 μ L of prepared NeuralX Medium one additional time for a total of 3 media changes. The final working volume will be approximately 50 μ L per well.

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

16. Transfer the plate(s) to a cell culture incubator set to 37°C, 5% CO₂, and 95% to 98% humidity, immediately after the final media change. Incubate for 2 days until the next media change.
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Subsequent Media Changes

17. Culture plates for a minimum of one-week post shipment to allow for full cell recovery.
18. Warm an aliquot of prepared NeuralX Medium to room temperature prior to each media exchange.
19. Perform half media changes every other day by aspirating 25 µL of old media and adding 25 µL of prepared NeuralX Medium (Table 1) to each well.

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

Perform Assay

20. After a minimum of one week in culture, the StemoniX microBrain 3D Assay Ready 384-Well Plate(s) are ready for experimentation.
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Please direct any questions or comments to Customer Service at
1-855-783-6669 ext.3, or customerservice@stemonix.com.

THANK YOU

Appendix A: Automated liquid handler setup information

Automated liquid handler (recommended method):

- A1. Establish the aspiration protocol **before** changing media in the microBrain 3D plates.
- 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 μ L 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 μ L.
- A5. Test the protocol and ensure 25 μ L of fluid is aspirated then dispensed into each well.

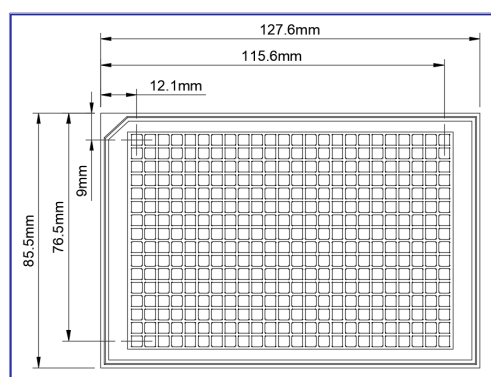


Figure 3. Corning Spheroid Microplate Measurements (Catalog Number 4516). Overhead view showing relevant width and length dimensions.

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

Appendix B: Recommended BrainPhys™ Media Components

Table 2: Preparation of BrainPhys 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) | NA | 1X | SV30010 |

- B1. Mix components together to the final volumes/ concentrations listed in Table 2.
B2. Refer to the manufacturer's recommendations for stability and storage conditions.

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